L Number	Hits	Search Text	DB	Time stamp
1	6	(BSA or (bovine adj1 serum adj1 albumin)) near8 linker near20 covalent	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:13
2	10	(BSA or (bovine adj1 serum adj1 albumin)) near10 linker near20 covalent	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:15
3	241	(BSA or (bovine adj1 serum adj1 albumin)) near10 covalent	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:26
4	3	((BSA or (bovine adj1 serum adj1 albumin)) near10 covalent) same (immoboli$5 or attach)	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:16
5	326	(BSA or (bovine adj1 serum adj1 albumin)) near17 covalent	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:19
6	4	((BSA or (bovine adj1 serum adj1 albumin)) near17 covalent) same (coat or attach or immobolized or immobolizing)	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:21
7	14	((BSA or (bovine adj1 serum adj1 albumin)) near17 covalent) same (array or chip or sensor)	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:21
8	151	(BSA or (bovine adj1 serum adj1 albumin)) same covalent same (linkage or linker or adaptor)	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:27
9	336	(BSA or (bovine adj1 serum adj1 albumin)) near20 covalent	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:27
10	42	((BSA or (bovine adj1 serum adj1 albumin)) same covalent same (linkage or linker or adaptor)) and ((BSA or (bovine adj1 serum adj1 albumin)) near20 covalent)	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 09:27

L Number	Hits	Search Text	DB	Time stamp
1	166	(BSA or (bovine adj1 serum adj1 albumin)) same (covalently or covalent) same (schiff or transacyl or michael or amine or thiol)	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 12:55
2	0	(BSA or (bovine adj1 serum adj1 albumin)) same (covalently or covalent) same (schiff and transacyl and michael and amine and thiol)	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 12:56
3	48	(BSA or (bovine adj1 serum adj1 albumin)) same (covalently or covalent) same (schiff or transacyl or michael or amine or thiol) same (linker or linkage or linked or adaptor)	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 13:40
4	166	(BSA or (bovine adj1 serum adj1 albumin)) same (covalently or covalent) same (schiff or transacyl or michael or amine or thiol)	USPAT; US-PGPUB; EPO; DERWENT	2004/05/12 13:40

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specify a corrected file name or you can enter "IGNORE" to continue
accessing the remaining file names entered.
ENTER A FILE NAME OR (IGNORE):ignore
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                        ENTRY    SESSION
FULL ESTIMATED COST                                      0.21       0.21

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=> (bsa or (bovine serum albumin))(P)covalent(P)(linkage or linker or adaptor)
L1            2 FILE AGRICOLA
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'ALBUMIN))(P)COVALENT'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'COVALENT(P)(LINKAGE'
L2           23 FILE BIOTECHNO
L3            0 FILE CONFSCI
L4            0 FILE HEALSAFE
L5            0 FILE IMSDRUGCONF
L6            8 FILE LIFESCI
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'ALBUMIN))(P)COVALENT'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'COVALENT(P)(LINKAGE'
L7            0 FILE MEDICONF
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'ALBUMIN))(P)COVALENT'
PROXIMITY OPERATOR LEVEL NOT CONSISTENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'COVALENT(P)(LINKAGE'
L8           14 FILE PASCAL

TOTAL FOR ALL FILES
L9           47 (BSA OR (BOVINE SERUM ALBUMIN))(P) COVALENT(P)(LINKAGE OR LINKER
                OR ADAPTOR)

=> dup rem
ENTER L# LIST OR (END):19
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DUPLICATE IS NOT AVAILABLE IN 'IMSDRUGCONF, MEDICONF'.
ANSWERS FROM THESE FILES WILL BE CONSIDERED UNIQUE
PROCESSING COMPLETED FOR L9
L10 39 DUP REM L9 (8 DUPLICATES REMOVED)

=> l10 and (sensor or array or chip)
L11 2 S L10
L12 0 FILE AGRICOLA
L13 22 S L10
L14 0 FILE BIOTECHNO
L15 0 S L10
L16 0 FILE CONFSCI
L17 0 S L10
L18 0 FILE HEALSAFE
L19 0 S L10
L20 0 FILE IMSDRUGCONF
L21 6 S L10
L22 0 FILE LIFESCI
L23 0 S L10
L24 0 FILE MEDICONF
L25 9 S L10
L26 3 FILE PASCAL

TOTAL FOR ALL FILES
L27 3 L10 AND (SENSOR OR ARRAY OR CHIP)

=> d l27 ibib abs total

L27 ANSWER 1 OF 3 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2000-0452131 PASCAL
TITLE (IN ENGLISH): Investigation into immobilisation of lactate oxidase to improve stability
AUTHOR: LILLIS B.; GROGAN C.; BERNEY H.; LANE W. A.
CORPORATE SOURCE: Natl Microelectronics Research Cent, Cork, Ireland
SOURCE: Sensors and Actuators, B: Chemical, (2000), 68(1), 109-114, 15 refs.
Conference: Proceedings of Eurosensors XIII, The Hague, Neth, 12 Sep 1999-15 Sep 1999
ISSN: 0925-4005
DOCUMENT TYPE: Journal; Conference
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: Switzerland
LANGUAGE: English
AVAILABILITY: INIST-19425 B
AN 2000-0452131 PASCAL
AB Lactate oxidase (LOx) is an unstable enzyme. In this work, a variety of immobilisation techniques are investigated in an effort to improve the long-term stability of the enzyme. These include **covalent linkage** to two membrane types, encapsulation in a **BSA** gel and four different sol-gel matrices. The enzyme glucose oxidase (GOx) was also immobilised in the same sol-gel matrices. The methods were assessed for both activity and stability of the enzyme and the mechanical rigidity of the matrix. The **BSA** and sol-gels both formed physically robust enzyme layers. The enzyme retained its activity in the **BSA** gel for 20 days. Activity of the enzyme was much higher in the sol-gel matrices and remained stable for at least 55 days. Sol-gel processing conditions were also investigated.

L27 ANSWER 2 OF 3 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1999-0067486 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 1999 INIST-CNRS. All rights reserved.

TITLE (IN ENGLISH): In situ quartz crystal microbalance monitoring of Fab'-fragment binding to **linker** lipids in a phosphatidylcholine monolayer matrix. Application to immunosensors
AUTHOR: VIKHOLM I.; ALBERS W. M.; VAELIMAEKI H.; HELLE H. STROEVE Pieter (pref.)
CORPORATE SOURCE: Technical Research Centre of Finland, Chemical Technology, P.O. Box 14021, 33101 Tampere, Finland Center on Polymer and Macromolecular Assemblies, Department of Chemical Engineering and Materials Science, University of California, Davis, CA, United States
SOURCE: Thin solid films, (1998), 327-29, 643-646, 18 refs. Conference: 8 International Conference on Organized Molecular Films, Pacific Grove CA (United States), 24 Aug 1997
ISSN: 0040-6090 CODEN: THSFAP
DOCUMENT TYPE: Journal; Conference
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: Switzerland
LANGUAGE: English
AVAILABILITY: INIST-13597, 354000071007471450
AN 1999-0067486 PASCAL

AB **Linker** lipids were embedded in a phosphatidylcholine monolayer matrix prepared at the air-water interface. The **covalent** coupling of antibody fragments, non-specific adsorption of **bovine serum albumin** and specific binding of antibodies was monitored in situ with a 10-MHz quartz crystal microbalance. The attachment of antibody fragments and the activity of the layers was also showed with standardized radioimmunoassay. The results demonstrate that the coupling of Fab'-fragments to **linker** lipids in a monolayer matrix is a promising approach to achieve a highly oriented layer of antibody fragments with a high density of binding sites on the **sensor** surface for immunological measurements.

L27 ANSWER 3 OF 3 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1998-0442687 PASCAL

TITLE (IN ENGLISH): Oriented immobilization of antibodies for immunosensing
AUTHOR: VIKHOLM I.; ALBERS W. M.
CORPORATE SOURCE: Technical Research Centre of Finland, Chemical Technology, P.O. Box 14021, 33101 Tampere, Finland
SOURCE: Langmuir, (1998), 14(14), 3865-3872, 52 refs.
ISSN: 0743-7463 CODEN: LANGD5
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-20642, 354000077129050200
AN 1998-0442687 PASCAL

AB The **covalent** coupling of antibody fragments to **linkers** embedded in a monolayer matrix of phosphatidylcholine and cholesterol was examined at the air-water interface by the means of a quartz crystal microbalance, QCM. Two **linkers** that bind the free thiols of the Fab' fragment were investigated. The nonspecific binding of **bovine serum albumin** and the specific binding of antigen were also monitored with the QCM. Standardized radioimmunoassay was used to confirm the immunoreaction and determine binding parameters. The monolayer formation of the **linker**

lipids in the ternary system of phosphatidylcholine and cholesterol was, moreover, demonstrated by film balance studies. The results demonstrate that the **covalent** coupling of Fab' fragments to linking groups embedded in a phospholipid monolayer matrix is a promising approach to achieve a defined immobilization of antibodies at the **sensor** surface with high antigen binding effeciency.

=> d l9 ibib abs total

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ACCESSION NUMBER: 92:76467 AGRICOLA
DOCUMENT NUMBER: IND92044648
TITLE: An approach for the stable immobilization of proteins.
AUTHOR(S): Leckband, D.; Langer, R.
CORPORATE SOURCE: Massachusetts Institute of Technology, Cambridge, MA
AVAILABILITY: DNAL (381 J8224)
SOURCE: Biotechnology and bioengineering, Feb 5, 1991. Vol. 37, No. 3. p. 227-237
Publisher: New York, N.Y. : John Wiley & Sons.
CODEN: BIBIAU; ISSN: 0006-3592
NOTE: Includes references.
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB An approach is presented for the stable **covalent** immobilization of proteins with a high retention of biological activity. First, chemical modification studies were used to establish enzyme structural and functional properties relevant to the **covalent** immobilization of an enzyme to agarose based supports. Heparinase was used as a model enzyme in this set of studies. Amine modifications result in 75-100% activity loss, but the effect is moderated by a reduction in the degree of derivatization. N-hydroxysuccinimide, 1,1, 1-trifluoroethanesulfonic acid, and epoxide activated agarose were utilized to determine the effect of amine reactive supports on immobilized enzyme activity retention. Cysteine modifications resulted in 25-50% loss in activity, but free cysteines were inaccessible to either immobilized bromoacetyl or p-chloromercuribenzoyl groups. Amine reactive coupling chemistries were therefore utilized for the **covalent** immobilization of heparinase. Second, to ensure maximal stability of the immobile protein-support **linkage**, the identification and subsequent elimination of the principal sources of protein detachment were systematically investigated. By using high-performance liquid chromatography (HPLC), electrophoresis, and radiolabeling techniques, the relative contributions of four potential detachment mechanisms-support degradation, proteolytic degradation, desorption of noncovalently bound protein, and bond solvolysis-were quantified. The mechanisms of lysozyme, **bovine serum albumin**, and heparinase leakage from N-hydroxysuccinimide or 1,1,1-trifluoroethanesulfonic acid activated agarose were elucidated. By use of stringent postimmobilization support wash procedures, noncovalently bound protein was shown to be the predominant source of immobilized protein loss. An effective postimmobilization washing procedure is presented for the removal of adsorbed protein and the complete elimination of immobilized protein loss.

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ACCESSION NUMBER: 92:53301 AGRICOLA
DOCUMENT NUMBER: IND92028333

TITLE: The galactose-binding sites of the cytotoxic lectin ricin can be chemically blocked in high yield with reactive ligands prepared by chemical modification of glycopeptides containing triantennary N-linked oligosaccharides.
AUTHOR(S): Lambert, J.M.; McIntyre, G.; Gauthier, M.N.; Zullo, D.; Rao, V.; Steeves, R.M.; Goldmacher, V.S.; Blattler, W.A.
CORPORATE SOURCE: ImmunoGen Inc., Cambridge, MA
AVAILABILITY: DNAL (381 B523)
SOURCE: Biochemistry, Apr 2, 1991. Vol. 30, No. 13. p. 3234-3247
Publisher: Washington, D.C. : American Chemical Society.
CODEN: BICHAW; ISSN: 0006-2960
NOTE: Includes references.
DOCUMENT TYPE: Article
FILE SEGMENT: U.S. Imprints not USDA, Experiment or Extension
LANGUAGE: English

AB A glycopeptide containing a triantennary N-linked oligosaccharide from fetuin was modified by a series of chemical and enzymic reactions to afford a reagent that contained a terminal residue of 6-(N-methylamino)-6-deoxy-D-galactose on one branch of the triantennary structure and terminal galactose residues on the other two branches. Binding assays and gel filtration experiments showed that this modified glycopeptide could bind to the sugar-binding sites of ricin. The ligand was activated at the 6-(N-methylamino)-6-deoxy-D-galactose residue by reaction with cyanuric chloride. The resulting dichlorotriazine derivative of the ligand reacts with ricin, forming a stable **covalent linkage**. The reaction was confined to the B-chain and was inhibited by lactose. **Bovine serum albumin** and ovalbumin were not modified by the activated ligand under similar conditions, and we conclude, therefore, that the reaction of the ligand with ricin B-chain was dependent upon specific binding to sugar-binding sites. Ricin that had its galactose-binding sites blocked by the **covalent** reaction with the activated ligand was purified by affinity chromatography. The major species in this fraction was found to contain 2 covalently linked ligands per ricin B-chain, while a minor species contained 3 ligands per B-chain. The cytotoxicity of blocked ricin was at least 1000-fold less than that of native ricin for cultured cells in vitro, even though the activity of the A-chain in a cell-free system was equal to that from native ricin. Modified ricin that contained only 1 covalently linked ligand was also purified. This fraction retained an ability to bind to galactose affinity columns, although with a lower affinity than ricin, and was only 5- to 20-fold less cytotoxic than native ricin.

L9 ANSWER 3 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 2003:37409669 BIOTECHNO
TITLE: **Covalent** Linking of Proteins and Cytokines to Suture: Enhancing the Immune Response of Head and Neck Cancer Patients
AUTHOR: Shibuya T.Y.; Kim S.; Nguyen K.; Parikh P.; Wadhwa A.; Brockardt C.; Do J.
CORPORATE SOURCE: Dr. T.Y. Shibuya, Dept. Otolaryngol./Head/Neck Surg., Univ. of CA Irvine Coll. of Medicine, Bldg. 25, 101 The City Drive South, Orange, CA 92868, United States. E-mail: tshibuya@uci.edu
SOURCE: Laryngoscope, (2003), 113/11 (1870-1884), 133 reference(s)
CODEN: LARYA8 ISSN: 0023-852X
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English

AN 2003:37409669 BIOTECHNO

AB Background: The immune system of advanced stage head and neck cancer patients is frequently suppressed. Poor immune function has been correlated with poor clinical outcome. Immunotherapeutic strategies have been previously attempted in an effort to enhance immune function and improve survival. Previous studies have shown surgical suture can be transformed into an immune stimulant capable of activating the T lymphocytes of cancer patients. The development of a process for covalently linking proteins and cytokines to suture could have enormous potential for the in vivo manipulation of the immune system. Hypothesis: We hypothesize proteins and cytokines can be covalently linked to surgical suture while preserving their functional properties. Study Design: Prospective study testing normal donor and head and neck squamous cell carcinoma (HNSCC) patient lymphocytes. Method: Polyester suture was acid hydrolyzed followed by reacting with 1-ethyl-3(-3-dimethylamino propyl carbodiimide) (EDAC) to create a suture-EDAC intermediate. Next, selected proteins (horseradish peroxidase [HRP] or **bovine serum albumin** [**BSA**]) or cytokines (interleukin [IL]-2 or interferon [IFN]-γ) were reacted with the suture-EDAC intermediate to test the **covalent linkage** of the selected protein or cytokine to suture. Functional activity of the linked proteins was measured spectrophotometrically. The linking of cytokines to suture was tested by stimulating normal donor peripheral blood lymphocytes (PBL) or HNSCC patients' lymphocytes. The functional activity was confirmed by proliferation, enzyme linked immunoadsorbent assay (ELISA), and phenotype expression of T cells. Results: The conditions for optimally linking a protein to polyester suture were defined using HRP as a model protein. HRP retained its enzymatic activity. The optimal conditions for linking IL-2 or IFN-γ were defined. The covalently linked cytokines retained their immune enhancing properties for stimulating PBL and lymph node lymphocytes (LNL) from HNSCC patients to proliferate, generate a T.sub.H1 immunologic profile of cytokines (IL-2, IL-12, IFN-γ), and stimulate T lymphocytes. Conclusion: This is the first report to demonstrate that cytokines can be covalently linked to surgical sutures and retain their immune-stimulating properties. Proteins linked to suture also retained their enzymatic activity. The clinical implications of functionally active cytokines or proteins linked to surgical suture may be very significant in the future for manipulating the immune system in vivo or enhancing wound healing.

L9 ANSWER 4 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN

ACCESSION NUMBER: 2002:34107364 BIOTECHNO

TITLE: Disulfide bond formation through Cys186 facilitates functionally relevant dimerization of trimeric hyaluronan-binding protein 1 (HABP1)/p32/gC1qR

AUTHOR: Jha B.K.; Salunke D.M.; Datta K.

CORPORATE SOURCE: K. Datta, Biochemistry Laboratory, School of Environmental Sciences, Jawaharlal Nehru University, New Delhi-110 067, India.
E-mail: datta_k@hotmail.com

SOURCE: European Journal of Biochemistry, (2002), 269/1 (298-306), 36 reference(s)
CODEN: EJBCAI ISSN: 0014-2956

DOCUMENT TYPE: Journal; Article

COUNTRY: United Kingdom

LANGUAGE: English

SUMMARY LANGUAGE: English

AN 2002:34107364 BIOTECHNO

AB Hyaluronan-binding protein 1 (HABP1), a ubiquitous multifunctional protein, interacts with hyaluronan, globular head of complement component 1q (gC1q), and clustered mannose and has been shown to be involved in cell signalling. In vitro, this recombinant protein isolated from human fibroblast exists in different oligomeric forms, as is evident from the results of various independent techniques in near-physiological

conditions. As shown by size-exclusion chromatography under various conditions and glutaraldehyde cross-linking, HABP1 exists as a noncovalently associated trimer in equilibrium with a small fraction of a covalently linked dimer of trimers, i.e. a hexamer. The formation of a covalently-linked hexamer of HABP1 through Cys186 as a dimer of trimers is achieved by thiol group oxidation, which can be blocked by modification of Cys186. The gradual structural transition caused by cysteine-mediated disulfide **linkage** is evident as the fluorescence intensity increases with increasing Hg.sup.2.sup.+ concentration until all the HABP1 trimer is converted into hexamer. In order to understand the functional implication of these transitions, we examined the affinity of the hexamer for different ligands. The hexamer shows enhanced affinity for hyaluronan, gC1q, and mannosylated **BSA** compared with the trimeric form. Our data, analyzed with reference to the HABP1/p32 crystal structure, suggest that the oligomerization state and the compactness of its structure are factors that regulate its function.

L9 ANSWER 5 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 2001:32435559 BIOTECHNO
TITLE: Stabilization of penicillin V acylase from Streptomyces lavendulae by **covalent** immobilization
AUTHOR: Torres-Bacete J.; Arroyo M.; Torres-Guzman R.; De La Mata I.; Castillon M.P.; Acebal C.
CORPORATE SOURCE: M. Arroyo, Dept. de Bioquim. y Biol. Molec. I, Facultad de Ciencias Biologicas, Universidad Complutense de Madrid, 28040 Madrid, Spain. E-mail: arroyo@solea.quim.ucm.es
SOURCE: Journal of Chemical Technology and Biotechnology, (2001), 76/5 (525-528), 26 reference(s) CODEN: JCTBDC ISSN: 0268-2575
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 2001:32435559 BIOTECHNO
AB Penicillin V acylase from the actinomycete Streptomyces lavendulae ATCC 13664 has been immobilized to epoxy-activated acrylic beads (Eupergit C®) by **covalent** binding. Further **linkage** of **bovine serum albumin** after enzyme immobilization was carried out in order to remove the remaining oxirane groups of the support. The obtained immobilized biocatalyst displayed double exponential deactivation kinetics at temperatures below 55°C, while the native enzyme followed single exponential decay at the same temperatures. We concluded that soluble penicillin acylase was deactivated in one step, whereas the immobilized enzyme showed an enzymatic intermediate state which is highly thermostable. As a consequence of the immobilization process, the enzyme displayed a 10-fold increase in its half-life at 40°C. At this temperature, the enzymatic intermediate state was progressively destabilized as the pH of the medium was increased. Thus, the optimum pH range for the immobilized enzyme preparation was established as being from 7.0 to 8.0. Higher pH values led to quicker enzyme deactivation. .COPYRGT. 2001 Society of Chemical Industry.

L9 ANSWER 6 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1999:29421856 BIOTECHNO
TITLE: Highly efficient immobilisation of antibody fragments to functionalised lipid monolayers
AUTHOR: Vikholm I.; Viitala T.; Albers W.M.; Peltonen J.
CORPORATE SOURCE: I. Vikholm, VTT Chemical Technology, P.O. Box 14021, FIN-33101 Tampere, Finland.
SOURCE: Biochimica et Biophysica Acta - Biomembranes, (1999),

1421/1 (39-52), 46 reference(s)
CODEN: BBBMBS ISSN: 0005-2736
PUBLISHER ITEM IDENT.: S0005273699001121
DOCUMENT TYPE: Journal; Article
COUNTRY: Netherlands
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1999:29421856 BIOTECHNO
AB The **covalent** attachment of Fab' fragments of polyclonal anti-human IgG to a lipid with a terminal **linker** group was examined by means of quartz crystal microbalance and surface plasmon resonance measurements. The **linker** lipid was embedded in binary or ternary monolayers of dipalmitoylphosphatidylcholine (DPPC) and cholesterol. Atomic force microscopy images of the films deposited on silanised SiO.sub.2 substrates showed that Fab' fragments take a standing position, thus giving site-directed immobilisation. Human IgG forms a network on interaction with the antibodies. Non-specific binding of **bovine serum albumin** was found to be very low when DPPC was used as the host matrix. At an optimal Fab' fragment concentration a binding capacity above 60% was obtained. However, if the surface concentration of the immobilised antibodies was too high, the binding capacity decreased due to steric hindrance. The results demonstrate that the **covalent** coupling of Fab' fragments to N-(ε-maleimidocaproyl)-dipalmitoylphosphatidylethanolamine (DPPE-EMC) embedded in a host monolayer matrix of DPPC is a promising approach to achieve a site-directed immobilisation of antibodies with high antigen-binding efficiency. Copyright (C) 1999 Elsevier Science B.V.

L9 ANSWER 7 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1998:28357927 BIOTECHNO
TITLE: **Covalent linkage** of recombinant hirudin to a novel ionic poly(carbonate) urethane polymer with protein binding sites: Determination of surface antithrombin activity
AUTHOR: Phaneuf M.D.; Szycher M.; Berceli S.A.; Dempsey D.J.; Quist W.C.; LoGerfo F.W.
CORPORATE SOURCE: M.D. Phaneuf, B. I. Deaconess Med. Ctr.-W. Campus, Vascular Surgery Research, Harvard Institutes of Med. Building, Boston, MA 02115, United States.
SOURCE: Artificial Organs, (1998), 22/8 (657-665), 24 reference(s)
CODEN: ARORD7 ISSN: 0160-564X
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28357927 BIOTECHNO
AB Surface thrombus formation on implantable biomaterials such as polyurethane is a major concern when utilizing these materials in the clinical setting. Thrombin, which is responsible for thrombus formation and smooth muscle cell activation, has been the target of numerous surface modification strategies in an effort to prevent this phenomenon from occurring. The purpose of this study was to covalently immobilize the potent, specific antithrombin agent recombinant hirudin (rHir) onto a novel polyurethane polymer synthesized with carboxylic acid groups which served as protein attachment sites. The in vitro efficacy of thrombin inhibition by this novel biomaterial surface was then evaluated. **Bovine serum albumin** (**BSA**), which was selected as the basecoat protein, was reacted with sulfo- SMCC in a 1:50 molar ratio. This **BSA**-SMCC complex was then covalently linked to the carboxylated polyurethane (cPU) surface via the crosslinker EDC (cPU- **BSA**-SMCC). This cPU-**BSA**-SMCC surface was then reacted with Traut's-modified .sup.1.sup.2.sup.5I-rHir, a procedure which created free sulfhydryl groups on rHir (cPU- **BSA**

-SMCC-S-.sup.1.sup.2.sup.5I-rHir). Using these crosslinking procedures, the cPU-**BSA**- SMCC-S-.sup.1.sup.2.sup.5I-rHir segments bound 188 ± 40 ng/cm.sup.2 (n = 60) whereas the controls with non specifically bound .sup.1.sup.2.sup.5I-rHir (Mitrathane + EDC + **BSA** + .sup.1.sup.2.sup.5I-rHir-SH and cPU-**BSA** + .sup.1.sup.2.sup.5I-rHir-SH) bound 13 ± 8 ng/cm.sup.2 and 4 ± 8 ng/cm.sup.2, respectively. Evaluation of these cPU-**BSA**-SMCC-S-.sup.1.sup.2.sup.5I-rHir segments for .sup.1.sup.3.sup.1I-thrombin inhibition using a chromogenic assay for thrombin showed that a maximum of 2.64 NIHU thrombin was inhibited in contrast to the controls which inhibited 0.76 and 0.70 NIHU. Controls with nonspecifically bound .sup.1.sup.2.sup.5I-rHir also had 0.31 and 0.76 NIHU .sup.1.sup.3.sup.1I-thrombin adherent to their respective surfaces whereas the maximum .sup.1.sup.3.sup.1I- thrombin binding to the cPU-**BSA** -SMCC-S-rHir segments was 1.51 NIHU. Exposure to .sup.1.sup.3.sup.1I-thrombin did not result in any release of covalently bound .sup.1.sup.2.sup.5I-rHir from the cPU-**BSA**-SMCC-S-.sup.1.sup.2.sup.5I-rHir segments. Thus, these results demonstrate that rHir can be covalently bound to this novel polyurethane surface and still maintain potent antithrombin activity.

L9 ANSWER 8 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1998:28226541 BIOTECHNO
TITLE: Removal of **bovine serum albumin** from cow's milk using chicken egg-yolk antibodies immobilized on chitosan gel
AUTHOR: Losso J.N.; Vanderstoep J.; Nakai S.
CORPORATE SOURCE: J.N. Losso, Department of Food Science, University of British Columbia, 6650 NW Marine Drive, Vancouver, BC V6T 1Z4, Canada.
E-mail: jlosso@unixg.ubc.ca
SOURCE: Food and Agricultural Immunology, (1998), 10/1 (47-56), 19 reference(s)
CODEN: FAIMEZ ISSN: 0954-0105
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28226541 BIOTECHNO
AB Polyclonal chicken antibodies raised against **bovine serum albumin** (**BSA**) were immobilized on chitosan gel for the immunoaffinity isolation of **BSA** from cow's milk. Antibodies (IgY) against **BSA** were isolated from egg-yolk, purified and antibody reactivity to antigen was measured. lgY developed against **BSA** was reduced by 2-mercaptoethylamine. The reactivities of reduced and whole IgY against **BSA** were not significantly different. The reduced IgY was covalently linked to chitosan gel through stable **covalent** thioether **linkages** using sulfo-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) as a cross-**linker**. The density of antibody IgY immobilized on chitosan gel was approximately 3-5 mg per ml of chitosan gel. The ligand-binding capacity of immobilized IgY towards **BSA** was 0.35-0.44 mg **BSA** per ml of chitosan gel. A single pass of skimmed milk through the column allowed the removal of **BSA** from the milk sample. The milk sample was analyzed, before and after immunoaffinity separation, by SDS-PAGE. **BSA** was desorbed with 0.5 M-glycine-HCl buffer at pH 2.8 but the reusability of the column was limited to three cycles. Alternatively, **BSA** was desorbed with 0.5 M-glycine-HCl buffer containing 2 M-NaCl at pH 4.6 after longer incubation times at a slower flow rate. The low ligand-binding capacity was not an impedement to reuse of the column. The column was reused more than 20 times with minimal loss of binding capacity.

L9 ANSWER 9 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN

ACCESSION NUMBER: 1998:28052865 BIOTECHNO
TITLE: Antibodies against EMC virus RNA-VPg recognize Tyr-(5'P → O)-pU and immunostain infected cells
AUTHOR: Bordunova O.A.; Turina O.V.; Nadezhdina E.S.; Shatskaya G.S.; Veiko V.P.; Drygin Y.F.
CORPORATE SOURCE: Y.F. Drygin, A.N. Belozersky Institute, Physico-Chemical Biology, Moscow State University, Khokhlov St., 119899 Moscow, Russian Federation. E-mail: drygin@nucleo.genebee.msu.su
SOURCE: FEBS Letters, (23 JAN 1998), 422/1 (57-60), 20 reference(s)
CODEN: FEBLAL ISSN: 0014-5793
PUBLISHER ITEM IDENT.: S0014579397016013
DOCUMENT TYPE: Journal; Article
COUNTRY: Netherlands
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1998:28052865 BIOTECHNO
AB **Covalent** complexes of nucleic acids and proteins are widespread among viruses. **Covalent** complexes of RNA and proteins are proposed to exist in eukaryotic cells. The goal of this work was to obtain specific antibodies to the **covalent linkage** unit (CLU) between virus RNA and protein to search cellular RNA-protein complexes. Antibodies were generated by direct immunization of a rabbit with the **BSA**-coupled EMC virus RNA-VPg complex. By a dot-blot immunoassay and immunofluorescent microscopy it was found that the antibodies specifically recognize both EMC virus RNA-VPg and synthetic CLU-containing compounds. Thus, a fraction of the antibodies was directed to CLU.

L9 ANSWER 10 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1997:27197482 BIOTECHNO
TITLE: **Covalent linkage** of recombinant hirudin to poly(ethylene terephthalate) (Dacron): Creation of a novel antithrombin surface
AUTHOR: Phaneuf M.D.; Berceli S.A.; Bide M.J.; Quist W.C.; LoGerfo F.W.
CORPORATE SOURCE: M.D. Phaneuf, Deaconess Hospital, Harvard Medical School, Vascular Surgery Research, 12 Blackfan Street, Boston, MA 02215, United States.
SOURCE: Biomaterials, (1997), 18/10 (755-765), 49 reference(s)
CODEN: BIMADU ISSN: 0142-9612
PUBLISHER ITEM IDENT.: S0142961296001937
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1997:27197482 BIOTECHNO
AB Thrombus formation and intimal hyperplasia on the surface of implantable biomaterials such as poly(ethylene terepthalate) (Dacron) vascular grafts are major concerns when utilizing these materials in the clinical setting. Thrombin, a pivotal enzyme in the blood coagulation cascade primarily responsible for thrombus formation and smooth muscle cell activation, has been the target of numerous strategies to prevent this phenomenon from occurring. The purpose of this study was to covalently immobilize the potent, specific antithrombin agent recombinant hirudin (rHir) to a modified Dacron surface and characterize the in vitro efficacy of thrombin inhibition by this novel biomaterial surface. **Bovine serum albumin** (**BSA**), which was selected as the 'basecoat' protein, was reacted with various molar ratios of the cross-**linker** sulphosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulpho-SMCC; 1:5-1:50). These **BSA**-SMCC complexes were then covalently linked to sodium hydroxide-hydrolysed Dacron (HD) segments via the cross-**linker**

1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). **Covalent linkage** of these complexes to HD (HD-**BSA**-SMCC) was not affected by any of the sulpho-SMCC cross-**linker** ratios assayed. rHir, which was initially reacted with 2-iminothiolane hydrochloride (Traut's reagent) in order to create sulphydryl groups, was then covalently bound to these HD-**BSA**-SMCC surfaces (HD-**BSA**-SMCC-S-rHir). The 1:50 (**BSA**: sulpho-SMCC) HD-**BSA**-SMCC-S-rHir segments bound 22-fold more rHir (111 ng per mg Dacron) compared to control segments and also possessed the greatest thrombin inhibition of the segments evaluated using a chromogenic substrate assay for thrombin. Further characterization of the HD-**BSA**-SMCC-S-rHir segments demonstrated that maximum thrombin inhibition was 20.43 NIHU, 14.6-fold greater inhibition than control segments (1.4 NIHU). Thrombin inhibition results were confirmed by .sup.1.sup.2.sup.5I-thrombin binding experiments, which demonstrated that the 1:50 HD-**BSA**-SMCC-S-rHir segments had significantly greater specific thrombin adhesion compared to control segments. Non-specific .sup.1.sup.2.sup.5I-thrombin binding to and release from the 1:50 HD-**BSA**-SMCC-S-rHir segments was also significantly less than the control segments. Thus, these results demonstrate that rHir can be covalently bound to a clinically utilized biomaterial (Dacron) while still maintaining its ability to bind and inhibit thrombin.

L9 ANSWER 11 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN

ACCESSION NUMBER: 1996:26388896 BIOTECHNO

TITLE: **Covalent** complexes between serum albumin and 7-hydroxycoumarin-4-acetic acid: Synthesis and applications in the spectrophotometric detection of long-chain fatty acids

AUTHOR: Demant E.J.F.

CORPORATE SOURCE: Dept. Medical Biochemistry/Genetics, The Panum Institute, University of Copenhagen, Blegdamsvej 3,DK-2200 Copenhagen N, Denmark.

SOURCE: Biochimica et Biophysica Acta - Lipids and Lipid Metabolism, (1996), 1304/1 (43-55)
CODEN: BBLLA6 ISSN: 0005-2760

PUBLISHER ITEM IDENT.: S0005276096001063

DOCUMENT TYPE: Journal; Article

COUNTRY: Netherlands

LANGUAGE: English

SUMMARY LANGUAGE: English

AN 1996:26388896 BIOTECHNO

AB Using a hydrophobic 8-aminooctanoic acid cross-**linker**, the pH-indicator dye 7-hydroxycoumarin-4-acetic acid (7-HCA) is covalently bound to **bovine serum albumin** (**BSA**) at the positions of reactive amino groups. A highly stable and water-soluble complex (**BSA**-HCA) with a 1:4 molar stoichiometry is synthesized. Appearance of a strong absorption band at λ(max) = 372 nm is associated to ionization of the 7-HCA chromophore when it is transferred from water into a basic microenvironment on the **BSA** surface. This particular surface site is related to the region(s) for high-affinity binding of long-chain fatty acids (FA). **BSA**-HCA responds to binding of FA (14-20 carbons) with immediate spectral changes and a decrease in 372 nm absorption, **BSA**-HCA provides an indicator-protein having a range of practical applications for the quantitative determination of long-chain FA in biochemical studies. The lower detection limit in a spectrophotometric method is .sim. 1 μM FA. **BSA**-HCA is usable both in various buffers and in the presence of detergents such as n-octylglucoside, Triton X-100 and CHAPS. A novel method for continuous assay of phospholipase A.sub.2 activity with **BSA**-HCA and a mixed phosphatidylcholine/CHAPS micellar substrate is reported.

L9 ANSWER 12 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1996:26379413 BIOTECHNO
TITLE: Shared reaction in solid-phase immunoassay for estriol determination
AUTHOR: Podesta A.; Smith C.J.; Villani C.; Montagnoli G.
CORPORATE SOURCE: DABFV, Universita di Pisa, Viale delle Piagge 2,56124 Pisa, Italy.
SOURCE: Steroids, (1996), 61/11 (622-626)
CODEN: STEDAM ISSN: 0039-128X
PUBLISHER ITEM IDENT.: S0039128X96001232
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1996:26379413 BIOTECHNO
AB In the search of factors responsible for the experimental difficulties in developing accurate and sensitive solid-phase immunoassay of steroids, an experimental model has been set up for the study of nonspecific interaction of the steroid analyte with the coating protein. Along with the development of a highly sensitive enzyme-linked, solid-phase immunoassay for estriol measurement, we observed evidence of shared reactions. This property, to our knowledge not previously described for monomeric, low- molecular-weight antigens like estrogens, has been attributed to the presence of **bovine serum albumin**, which is capable of binding estrogens through hydrophobic interactions. The addition of estriol in solution in large excess did not reach a complete inhibition of the binding, so the possibility was excluded that the antibody simply binds to the adsorbed estrogen. The simplest explanation for the occurrence of the reaction is the hypothesis that a family of antigen determinants arises when the estriol is conjugated to a protein carrier. The corresponding antibodies are revealed only when the estrogen participates to the actual analytical system in the form of a steroid-protein conjugate. In the experiment, the estriol has been recognized as being coupled with one or more amino acid side chains present around its site of **covalent linkage** to the immunogen protein. The discussed results may be of help in developing a solid-phase immunoassay of small antigens as steroids, but also in applying the hybridoma and phage display technologies, the screening methods of which are based on sensitized solid phases.

L9 ANSWER 13 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1995:26031773 BIOTECHNO
TITLE: Prolonged circulation of recombinant human granulocyte-colony stimulating factor by **covalent linkage** to albumin through a heterobifunctional polyethylene glycol
AUTHOR: Paige A.G.; Whitcomb K.L.; Liu J.; Kinstler O.
CORPORATE SOURCE: Amgen Inc., 1840 DeHavilland Drive,Thousand Oaks, CA 91320, United States.
SOURCE: Pharmaceutical Research, (1995), 12/12 (1883-1888)
CODEN: PHREEB ISSN: 0724-8741
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1995:26031773 BIOTECHNO
AB Purpose. Recombinant human granulocyte-colony stimulating factor (rhG-CSF) was covalently conjugated to both rat and human serum albumin (RSA and HSA respectively) to increases the circulating half life (t(.half.)) of rhG-CSF. Methods. Conjugates of RSA (MW 67,000) and HSA (MW 66,000) were prepared by linking the two proteins through a heterobifunctional maleimido-carboxyl polyethylene glycol (PEG) and were tested in the rat. The conjugates were injected intravenously (IV) at the equivalent dose of 50 μg/kg of rhG-CSF, and white blood cell (WBC)

counts and plasma concentrations of drug were determined. A comparison of pharmacokinetic parameters was made between rhG-CSF, the conjugates RSA-PEG-rhG-CSF and HSA-PEG-rhG-CSF, and a non-**covalent** mixture of rhC-CSF and HSA. Results. The albumin-rhG-CSF conjugates are eliminated more slowly from the circulation. The clearance values are reduced from 0.839 ± 0.121 ml/min/kg for rhG-CSF to 0.172 ± 0.013 ml/min/kg for RSA-PEG-rhG-CSF and 0.141 ± 0.005 ml/min/kg for HSA-PEG-rhG-CSF. WBC counts increased in both absolute number and duration as compared to rhG-CSF alone. The albumin rhC-CSF conjugates had enhanced serum stability relative to free rhG-CSF. The rate of degradation of the albumin conjugates incubated in rat serum at 37°C decreased five fold. Conclusions. The results from the study show that specific conjugation of rhG-CSF to albumin decreases plasma clearance in vivo, causes increased WBC response, and increases serum stability as compared to free rhG-CSF.

L9 ANSWER 14 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1993:23069779 BIOTECHNO
TITLE: Receptor-mediated antigen delivery into macrophages: Complexing antigen to α.sub.2-macroglobulin enhances presentation to T cells
AUTHOR: Chu C.T.; Pizzo S.V.
CORPORATE SOURCE: Department of Pathology, Duke University Medical Center, Box 3712,Durham, NC 27710, United States.
SOURCE: Journal of Immunology, (1993), 150/1 (48-58)
CODEN: JOIMA3 ISSN: 0022-1767
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1993:23069779 BIOTECHNO
AB Macrophages secrete α.sub.2-macroglobulin (α.sub.2M), a protein that may facilitate early Ag handling. α.sub.2M is able to entrap and form **covalent linkages** with diverse proteins during a transient proteinase-activated state. The resulting complexes are rapidly endocytosed after binding to high affinity receptors. Such a system could be capable of efficiently delivering a multitude of proteins to macrophages. We have used T hybridoma clones that respond only to hen egg lysozyme, in a MHC-restricted manner, to probe the effect of complex formation on Ag uptake and processing by murine macrophages. Radiolabeled lysozyme was internalized more rapidly and to a greater extent when bound to α.sub.2M than when unbound. Macrophages pulsed with lysozyme-α.sub.2M-elastase complexes required 200 to 250 times less Ag than those pulsed with free lysozyme to achieve effective presentation to T cells. Adding equimolar amounts of α.sub.2M-elastase complexes, or of α.sub.2M- methylamine, to free lysozyme had no effect on basal lysozyme presentation. Receptor-recognized forms of α.sub.2M, but not lysozyme or **BSA**, competed effectively for both uptake and presentation of lysozyme-α.sub.2M-elastase complexes. These results indicate that proteinase-activated α.sub.2M can enhance Ag processing by carrying Ag into macrophages through a receptor-mediated process.

L9 ANSWER 15 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1992:22262483 BIOTECHNO
TITLE: Binding of ethylenediamine to phosphatidylserine is inhibitory to Na.sup.+/K.sup.+-ATPase
AUTHOR: Schuurmans Stekhoven F.M.A.H.; Tesser G.I.; Ramsteyn G.; Swarts H.G.P.; De Pont J.J.H.H.M.
CORPORATE SOURCE: Department of Biochemistry, University of Nijmegen, P.O. Box 9101,6500 HB Nijmegen, Netherlands.
SOURCE: Biochimica et Biophysica Acta - Biomembranes, (1992), 1109/1 (17-32)
CODEN: BBBMBS ISSN: 0005-2736

DOCUMENT TYPE: Journal; Article
COUNTRY: Netherlands
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1992:22262483 BIOTECHNO
AB **Covalent linkage** of ethylenediamine with the Na.sup.+/K.sup.+-ATPase complex from rabbit kidney outer medulla by the use of the water-soluble carbodiimide, N-ethyl,N'-(3-dimethylaminopropyl)carbodiimide, resulted in a 73% reaction with phosphatidylserine and only 27% with carboxylic groups in the proteic component of the enzyme. Condensation products from the reaction between phosphatidylserine and ethylenediamine, N-(O-phosphatidylseryl)ethylenediamine, N,N,-bis(O-phosphatidylseryl)ethylenediamine and its intermediary product O-phosphatidyl-¢N,N'-bis(seryl)!ethylenediamine, were synthesised. Symmetrically substituted ethylenediamine was the most likely condensation product of ethylenediamine with endogenous phosphatidylserine. The synthesised lipids were incorporated in proteoliposomes containing Na.sup.+/K.sup.+-ATPase and only the addition of the phospholipid phosphatidylcholine. The ratio of phospholipid to protein was 52 (w/w). These proteoliposomes were perforated by the addition of 0.5% cholate and both the Na.sup.+-dependent phosphorylation level and its dependence on Na.sup.+, Mg.sup.2.sup.+ and ATP were measured. Phosphatidylcholine alone increased the half-maximal activation concentration for Na.sup.+ (¢Na.sup.+!.sub.0.sub...sub.5) from 0.2 to 1-2 mM, for Mg.sup.2.sup.+ from 0.1 to 0.8 μM and for ATP from 0.02 to 0.3 μM. The K(i) for K.sup.+ (in the absence of Na.sup.+) was unaffected: 12.8 μM vs. 12.5 μM in the non-reconstituted system. Replacing 10 mol% of phosphatidylcholine by phosphatidylethanolamine or phosphatidylserine had no significant effect on ¢Na.sup.+!.sub.0.sub...sub.5: 1.1 and 0.7 mM, respectively. Replacing 5 mol% phosphatidylcholine by the bis(phosphatidylseryl) substituent of ethylenediamine further increased ¢Na.sup.+!.sub.0.sub...sub.5 to 13.7 mM, while half-maximal activation concentrations for Mg.sup.2.sup.+ and ATP were unaltered. The mono-phosphatidylseryl derivatives of ethylenediamine, each 5 mol%, also increased ¢Na.sup.+!.sub.0.sub.5, but to a lesser extent (3.2-3.8 mM). In addition to their competitive effects, the phosphatidylseryl-substituted ethylenediamine compounds exerted a slowly-increasing non-competitive inhibition, not only in phosphorylation, but also in overall ATPase activity, which was reduced, although not abolished, by exogenous protein (**bovine serum albumin**). A detergent-like action in the usual sense is unlikely since liposomes containing these lipids remained intact. These studies prove that phospholipids are not only required for optimal activity of this transport enzyme, but in excess or in compositions deviating from the normal, may also be inhibitory.

L9 ANSWER 16 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1992:22043780 BIOTECHNO
TITLE: Method for analysis, and distribution profile, of covalently-linked ferritin-daunorubicin conjugate in the blood of trypanosome-infected mice
AUTHOR: Brown J.E.; Patterson L.H.; Williamson J.; Brown J.R.
CORPORATE SOURCE: Department of Pharmaceutical Chemistry, School of Pharmacy, University of Bradford, Bradford BD7 1DP, United Kingdom.
SOURCE: Journal of Pharmacy and Pharmacology, (1992), 44/1 (48-51)
CODEN: JPPMAB ISSN: 0022-3573
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English

AN 1992:22043780 BIOTECHNO
AB Daunorubicin is a highly potent trypanocide in-vitro but is inactive in-vivo. When daunorubicin is conjugated to **bovine serum albumin** or horse spleen ferritin using Schiffs base **linkages**, the complex is trypanocidal in-vitro and in-vivo. We have developed novel analytical methods, using HPLC with fluorimetric detection, for the quantitation of daunorubicin and doxorubicin in biological samples, either as unconjugated drug, or when covalently linked to macromolecules or particles. Ferritin-daunorubicin conjugate (25 mg kg.sup.-.sup.1) was administered intraperitoneally to mice infected with monomorphic Trypanosoma brucei rhodesiense; peak plasma levels occurred after 1.5 h, and were 5 times higher than those resulting from administration of an equivalent amount of unconjugated daunorubicin. Plasma levels then declined rapidly (t(.half.) for 1-6 h period was 0.58 and 0.86 h respectively for conjugated and unconjugated daunorubicin). However, higher plasma levels were seen 24 h after treatment, suggesting the distribution profile of daunorubicin when conjugated to ferritin is multiphasic with resultant high levels of daunorubicin obtained for a prolonged time period.

L9 ANSWER 17 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1991:21117433 BIOTECHNO
TITLE: An approach for the stable immobilization of proteins
AUTHOR: Leckband D.; Langer R.
CORPORATE SOURCE: Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139, United States.
SOURCE: Biotechnology and Bioengineering, (1991), 37/3 (227-237)
CODEN: BIBIAU ISSN: 0006-3592
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1991:21117433 BIOTECHNO
AB An approach is presented for the stable **covalent** immobilization of proteins with a high retention of biological activity. First, chemical modification studies were used to establish enzyme structural and functional properties relevant to the **covalent** immobilization of an enzyme to agarose based supports. Heparinase was used as a model enzyme in this set of studies. Amine modifications result in 75-100% activity loss, but the effect is moderated by a reduction in the degree of derivatization. N-hydroxysuccinimide, 1,1,1-trifluoroethanesulfonic acid, and epoxide activated agarose were utilized to determine the effect of amine reactive supports on immobilized enzyme activity retention. Cysteine modifications resulted in 25-50% loss in activity, but free cysteines were inaccessible to either immobilized bromoacetyl or p-chloromercuribenzoyl groups. Amine reactive coupling chemistries were therefore utilized for the **covalent** immobilization of heparinase. Second, to ensure maximal stability of the immobile protein-support **linkage**, the identification and subsequent elimination of the principal sources of protein detachment were systematically investigated. By using high-performance liquid chromatography (HPLC), electrophoresis and radiolabeling techniques, the relative contributions of four potential detachment mechanisms - support degradation, proteolytic degradation, desorption of noncovalently bound protein, and bond solvolysis - were quantified. The mechanisms of lysozyme, **bovine serum albumin**, and heparinase leakage from N-hydroxysuccinimide or 1,1,1-trifluoroethanesulfonic acid activated agarose were elucidated. By use of stringent postimmobilization support wash procedures, noncovalently bound protein was shown to be the predominant source of immobilized protein loss. An effective postimmobilization washing procedure is presented for the removal of adsorbed protein and the complete elimination of

immobilized protein loss.

L9 ANSWER 18 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1988:18238291 BIOTECHNO
TITLE: Immunopotentiation of the humoral response by liposomes: Encapsulation versus **covalent linkage**
AUTHOR: Shahum E.; Therien H.-M.
CORPORATE SOURCE: Department de Chimie-Biologie, Universite du Quebec a Trois-Rivieres, Trois-Rivieres, Que. G9A 5H7, Canada.
SOURCE: Immunology, (1988), 65/2 (315-317)
CODEN: IMMUAM ISSN: 0019-2805
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1988:18238291 BIOTECHNO
AB Two different modes of antigen association with liposomes were compared for their stimulation of IgM- and IgG-producing cells in primary- and secondary-response experiments. The study was carried out on BALB/c mice using the antigen **bovine serum albumin** either free, encapsulated in liposomes or covalently linked to the liposomal surface. Our results indicate that, although both types of liposome association are equally efficient in potentiating the humoral response, encapsulation mainly favours IgG isotype production with little or no effect on the IgM subset, while **covalent linkage** stimulates the production of both IgG and IgM. Our results reconcile some apparently conflicting published data and suggest that the mode of antigen association with liposomes considerably influences the pathways by which stimulation occurs.

L9 ANSWER 19 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1988:18072259 BIOTECHNO
TITLE: Radioimmunoassay for fluphenazine sulfoxide in human plasma
AUTHOR: Midha K.K.; Hawes E.M.; Hubbard J.W.; McKay G.; Rauw G.; Sardessai S.; Aravagiri M.; Moore M.D.
CORPORATE SOURCE: University of Saskatchewan College of Pharmacy, Saskatoon, Saskatchewan, Canada.
SOURCE: Journal of Pharmacological Methods, (1988), 19/1 (63-74)
CODEN: JPMED0 ISSN: 0160-5402
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1988:18072259 BIOTECHNO
AB Antisera to fluphenazine sulfoxide were raised in New Zealand white rabbits to an immunogen synthesized by **covalent linkage** of **bovine serum albumin** to 10-¢¢3-¢4-carboxybutyl)-1-piperazinyl!propyl!!-2-trifluoromethyl-10H-phenot hiazine 5-sulfoxide. With use of an antiserum, a radioimmunoassay for fluphenazine sulfoxide was developed that is able to quantitate 0.156 ng ml.sup.-.sup.1 using only a 200 μl plasma sample with a coefficient of variation < 5%. The antiserum had negligible cross-reactivities to fluphenazine (<1%) and its important metabolites, such as fluphenazine N.sup.4'-oxide (1%), 7-hydroxyfluphenazine (<1%), and N.sup.4'-deshydroxy-ethylfluphenazine (1%). The cross-reactivities with structurally similar phenothiazine 5-sulfoxides, such as those of trifluoperazine, prochlorperazine, perphenazine, and N.sup.4'-deshydroxyethylfluphenazine, were considerable, such that the antiserum can be used to develop a quantitative radioimmunoassay for any of these compounds. The reported radioimmunoassay was found to be suitable and adequate to quantitate fluphenazine sulfoxide in the plasma of patients

treated with oral or intramuscular fluphenazine.

L9 ANSWER 20 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1987:18006157 BIOTECHNO
TITLE: Mechanism of formation and quantitation of imines, pyrroles, and stable nonpyrrole adducts in 2,5-hexanedione-treated protein
AUTHOR: DeCaprio A.P.; Jackowski S.J.; Regan K.A.
CORPORATE SOURCE: Wadsworth Center for Laboratories and Research, New York State Department of Health, Empire State Plaza, Albany, NY 12201, United States.
SOURCE: Molecular Pharmacology, (1987), 32/4 (542-548)
CODEN: MOPMA3 ISSN: 0026-895X
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AN 1987:18006157 BIOTECHNO
AB The condensation of γ-diketones with protein ε-amino moieties to yield alkylpyrrole adducts has been demonstrated in many in vitro and in vivo systems, although certain features of this reaction remain unclear. The present in vitro study was designed to examine additional aspects of γ-diketone-protein interactions including the possible formation of imine intermediates and stable nonpyrrole products, and the potential for conformational changes in pyrrolylated protein. Values for total, stable **covalent** binding were consistently higher than p-dimethylaminobenzaldehyde (DMAB)-detectable pyrrole adduct concentrations when **bovine serum albumin** (**BSA**) was incubated (24 hr, 37°C) with ¢.sup.1.sup.4C!-2,5-hexanedione (2,5-HD) at diketone:lysine ratios >=5:1 (at pH 9.5) or 1:1 (at pH 7.4). Treatment of pyrrolylated **BSA** with proteases before the DMAB assay decreased but did not eliminate the difference between these parameters. Quantitative amino acid analysis of pyrrolylated **BSA** revealed molar decreases in lysine content equivalent to DMAB-detectable pyrrole adduct concentrations; no other amino acids were significantly altered. Cleavage of disulfide bonds in pyrrolylated **BSA** by dithiothreitol resulted in an apparent decrease in DMAB-detectable pyrrole, which was reversible upon subsequent protease treatment. A similar decrease was not seen with pyrrolylated concanavalin A, a protein that lacks disulfide **linkages**. Samples of **BSA** were incubated with ¢.sup.1.sup.4C!-2,5-hexanedione for 2-144 hr and a portion of each incubation mixture treated with NaCNBH.sub.3 to selectively reduce imines to stable amines. Substantial levels of an imine intermediate were detected at 2, 6, and 24 hr but not at 144 hr. The above findings support proposed mechanisms involving imine intermediates in the pyrrolylation reaction. In addition, evidence for the formation of stable nonpyrrole adducts at high diketone:amine molar ratios has been provided. Results consistent with potential conformational alterations in pyrrolylated protein have also been demonstrated.

L9 ANSWER 21 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1985:15229749 BIOTECHNO
TITLE: Suitable hollow fibre immunobioreactors for specific ex vivo removal of antibodies and antigens from plasma
AUTHOR: Larue C.; Gueraud V.; Rivat C.
CORPORATE SOURCE: INSERM Unite 78, 76230 Bois-Guillaume, France.
SOURCE: Clinical and Experimental Immunology, (1985), 62/1 (217-224)
CODEN: CEXIAL
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
AN 1985:15229749 BIOTECHNO

AB Studies were undertaken to detemine the applicability and effectiveness of a new immunoadsorbent, constituted of cellulose hollow fibres chemically modified (BrCN) to link selected proteins. The method has been assayed on a simple model of antibody elimination: myeloma IgG or **BSA** as antigens were covalently linked to cellulose; such an immunoadsorbent can selectively and efficiently deplete circulating antibodies in vitro and ex vivo (on immunized dogs) from whole blood, without releasing linked protein into the hosts' circulation. The original approach of using this method to remove antibodies has been extended to specifically remove antigens (for this purpose, antibodies were conjugated to cellulose), in order to investigate an immunoadsorption therapy in familial hypercholesterolemia, characterized by a plasmatic overload of low-density-lipoproteins (LDL), of which apolipoprotein B is the major protein. After **covalent linkage** of isolated anti-apolipoprotein B antibodies to cellulose, human plasma LDL levels were effectively and specifically reduced by this procedure.

L9 ANSWER 22 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1984:15202528 BIOTECHNO
TITLE: Radioimmunoassay for trimeprazine in human plasma
AUTHOR: McKay G.; Rauw G.A.J.; Stonkus M.D.; et al.
CORPORATE SOURCE: College of Pharmacy, University of Saskatchewan, Saskatoon, Sask., Canada.
SOURCE: Journal of Pharmacological Methods, (1984), 12/3 (203-211)
CODEN: JPMED0
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
AN 1984:15202528 BIOTECHNO
AB Antisera to trimeprazine were raised in New Zealand white rabbits to an immunogen synthesized by **covalent linkage** of **bovine serum albumin** to N-(2-carboxyethyl)desmethyltrimeprazine. By use of an antiserum, a radioimmunoassay for trimeprazine was developed that is able to quantitate 0.38 ng/ml.sup.-.sup.1 in a 200 μl plasma sample with a coefficient of variation of approximately 12%. The antiserum did not cross-react with the supposedly pharmacologically inactive metabolite trimeprazine sulfoxide; however, the cross-reactivity with the supposedly active metabolite N-desmethyltrimeprazine is significant (49%). The radioimmunoassay was able to measure the drug and/or N-desalkyl metabolites in plasma samples obtained as late as 24 hr following administration of a single oral dose (10 mg) of trimeprazine tartate. Analysis of the same plasma samples by a published high-performance liquid chromatographic procedure gave values much lower than those obtained by the radioimmunoassay, indicating the N-desalkyl metabolites are produced significantly after trimeprazine oral administration.

L9 ANSWER 23 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1985:15005829 BIOTECHNO
TITLE: **Covalent linkage** of .sup.1.sup.2.sup.5I-insulin to a cytosolic insulin-degrading enzyme
AUTHOR: Shii K.; Baba S.; Yokono K.; Roth R.A.
CORPORATE SOURCE: Department of Pharmacology, Stanford University School of Medicine, Stanford, CA 94305, United States.
SOURCE: Journal of Biological Chemistry, (1985), 260/11 (6503-6506)
CODEN: JBCHA3
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
AN 1985:15005829 BIOTECHNO

AB Cytosl extracts high in insulin-degrading activity were cross-linked to .sup.1.sup.2.sup.5I-insulin with the bifunctional cross-**linker** disuccinimidyl suberate. With cytosols from either rat muscle, liver, kidney or brain or human erythrocytes, only a single protein (M(r)=110,000) was specifically labeled. Three different lines of evidence indicated that this labeled protein is insulin-degrading enzyme, a cysteine protease which accounts for most of the insulin-degrading activity in cell extracts. Firstly, the cross-linking of .sup.1.sup.2.sup.5I-insulin to this protein is inhibited by unlabeled insulin over the same concentration range of insulin which inhibits degradation. Separated insulin A and B chain were less potent at inhibiting cross-linking, whereas **bovine serum albumin** and cytochrome c were without effect. Secondly, antibodies to purified insulin-degrading enzyme precipitated the labeled protein in parallel with their ability to precipitate the insulin-degrading activity of the extracts. Thirdly, when the insulin-degrading activity was purified 40,000-fold from erythrocytes, this M(r) 110,000 protein co-purified. These results indicate that cross-linking .sup.1.sup.2.sup.5I-insulin may be a convenient method for labeling the insulin-degrading enzyme.

L9 ANSWER 24 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1984:14058324 BIOTECHNO
TITLE: Inhibition of immune precipitation by complement
AUTHOR: Hong K.; Takata Y.; Sayama K.; et al.
CORPORATE SOURCE: Department of Bacteriology, Osaka University Medical School, Suita, Osaka 565, Japan.
SOURCE: Journal of Immunology, (1984), 133/3 (1464-1470)
CODEN: JOIMA3
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
AN 1984:14058324 BIOTECHNO
AB Normal human complement serum (NHS) inhibited precipitin reactions between tetanus toxoid and human or rabbit anti-tetanus toxoid IgG antibody, between **bovine serum albumin** (**BSA**) and rabbit anti-**BSA** IgG antibody, and between hen egg albumin and rabbit anti-egg albumin IgG antibody. Ethylene-diaminetetraacetic acid (EDTA) prevented this inhibition. Mg-ethyleneglycol-bis(aminoethyl)-tetraacetic acid-(EGTA) also prevented the inhibition except with lower concentrations of antibody and antigen. Therefore, the inhibition of immune precipitation seemed to occur mainly through the classical pathway of complement activation. The alternative pathway was usually dispensable, but it augmented the inhibition. Guinea pig complement serum (NGS) was less effective than NHS in inhibiting immune precipitation. Guinea pig serum deficient in C4 (C4DGS) did not inhibit the immune precipitation. Mouse complement serum was effective for inhibiting precipitation, and C5-deficient serum was as effective as normal serum. Therefore, the inhibition of immune precipitation is considered to occur by activation of complement up to the step of C3. The size of the soluble immune complexes formed in the presence of NHS varied depending on the concentrations of antibody and antigen, even when the ratio of antigen to antibody was constant. On incubation at 37° C immune precipitation was inhibited by 1/2 dilution of NHS for 2 to 3 hr and then gradually increased to the level in the absence of complement. When the immune complexes were formed in the presence of serum containing complement, fragments of C4 and C3 were incorporated into the soluble immune complexes. The C3 fragments incorporated into the soluble complexes were C3b, iC3B, C3c, and C3d, some of which were bound covalently with heavy chains of IgG antibody molecules. Some of the **covalent linkages** between C3 fragments and IgG seemed to be destroyed by alkali treatment, but not by hydroxylamine treatment. The formation of **covalent** bonds between IgG and C3 and probably C4 was essential for inhibition of immune precipitation, because

inhibitors of their formation, such as putrescine, cadaverine, and salicylhydroxamic acid, effectively prevented the inhibition of precipitation. When antigen and antibody reacted in the presence of mixtures of various combinations of isolated complement components, C1, C4, C2, and C3 showed maximal inhibition of immune precipitation, whereas factors I and H had little effect.

L9 ANSWER 25 OF 47 BIOTECHNO COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1983:13039863 BIOTECHNO
TITLE: Immune response mediated by liposome-associated protein antigens. III. Immunogenecitiy of **bovine serum albumins** covalently coupled to vesicle surface
AUTHOR: Shek P.N.; Heath T.D.
CORPORATE SOURCE: Immunol. Group, Health Sci. Sect., Biosci. Div., Dep. Natl. Def., Def. Civ. Inst. Environ. Med., Downsview, Ont. M3M 3B9, Canada.
SOURCE: Immunology, (1983), 50/1 (101-106)
CODEN: IMMUAM
DOCUMENT TYPE: Journal; Article
COUNTRY: United Kingdom
LANGUAGE: English
AN 1983:13039863 BIOTECHNO
AB A protein antigen, **bovine serum albumin** (**BSA**), was covalently linked to the surface of preformed large unilamellar vesicles composed of phosphatidylcholine, cholesterol, and N-¢4-(p-maleimidophenyl)butyryl!phosphatidylethanolamine (MPB-PE). The interaction between thiolated **BSA** and MPB-PE resulted in the production of a protein-liposome conjugate via the formation of an irreversible **covalent** bond. Mice immunized with liposome-coupled **BSA** were found to generate a vigorous **BSA**-specific plaque-forming cell (PFC) response. No significant response was observed in control animals given simultaneous, but separate injections of thiol-**BSA** and liposomes. Thus, there seems to be a need for successful and stable **linkage** between the antigen and the carrier. The elicitation of an optimal antigen-specific PFC response was also found to require the vesicle surface to be coated with a certain minimum distribution of the antigen. Results of this study demonstrate that the **covalent** coupling of a protein antigen to the liposome surface is very effective in potentiating the protein-specific antibody response and the immunogenicity of the conjugate is dependent on the epitope density of the antigen.

L9 ANSWER 26 OF 47 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 1999:96407 LIFESCI
TITLE: An immunochemical study on tau glycation in paired helical filaments
AUTHOR: Ko, L.W.; Ko, E.C.; Nacharaju, P.; Liu, W.K.; Chang, E.
CORPORATE SOURCE: Department of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY, 10461, USA
SOURCE: Brain Research [Brain Res.], (19990605) vol. 830, no. 2, pp. 301-313.
ISSN: 0006-8993.
DOCUMENT TYPE: Journal
FILE SEGMENT: N3
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Glycation is a non-enzymatic posttranslational modification that involves a **covalent linkage** between a sugar and an amino group of protein molecule forming ketoamine. Subsequent oxidation, fragmentation and/or crosslinking of ketoamine leads to the production of advanced glycation endproducts (AGEs). Formation of AGEs causes detrimental effects on the structure and function of affected proteins. Accumulation of AGEs has been implicated in normal aging and in the pathogenesis of

diabetes-associated complications and Alzheimer's disease (AD). Of all AGEs, N super(epsilon)-(carboxymethyl)lysine (CML) is a major glycoxidation product known to be stable and accumulate progressively in vivo. In order to determine if tau is glycated in AD, we raised a rabbit antibody to CML that demonstrated its usefulness in detecting glycation of different proteins in vitro, including **BSA**, ribonuclease, lysozyme and recombinant tau. Immunochemical analyses indicated that ribose and glucose-6-phosphate are more effective than glucose in generating CML formation in these proteins. We used this antibody to probe for glycation in the following human tau preparations: tau of normal brains and preparations of soluble PHF-tau as well as insoluble PHF from AD brains. All three principal tau components resolved from PHF-tau on Western blots showed CML immunoreactivity indicating that tau is glycated in PHF-tau; and insoluble PHF exhibited prominent CML immunoreactivity on top of the stacking gel. Moreover, immunoelectron microscopic analyses indicate that the anti-CML antibody labels predominantly PHF in aggregates. Taken together, these results suggest that tau becomes glycated in PHF-tau and glycation may play a role in stabilizing PHF aggregation leading to tangle formation in AD.

L9 ANSWER 27 OF 47 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 1999:81341 LIFESCI
TITLE: Opposite behaviors of reactive metabolites of tienilic acid and its isomer toward liver proteins: Use of specific anti-tienilic acid-protein adduct antibodies and the possible relationship with different hepatotoxic effects of the two compounds
AUTHOR: Bonierbale, E.; Valadon, P.; Pons, C.; Desfosses, B.; Dansette, P.M.*; Mansuy, D.
CORPORATE SOURCE: Laboratoire de Chimie et Biochimie Pharmacologiques et Toxicologiques, URA 400 CNRS, Universite Paris V, 45 rue des Saints-Peres, 75270 Paris Cedex 06, France; E-mail: dansette@citi2.fr
SOURCE: Chemical Research in Toxicology [Chem. Res. Toxicol.], (19990300) vol. 12, no. 3, pp. 286-296. ISSN: 0893-228X.
DOCUMENT TYPE: Journal
FILE SEGMENT: X
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Tienilic acid (TA) is responsible for an immune-mediated drug-induced hepatitis in humans, while its isomer (TAI) triggers a direct hepatitis in rats. In this study, we describe an immunological approach developed for studying the specificity of the **covalent** binding of these two compounds. For this purpose, two different coupling strategies were used to obtain TA-carrier protein conjugates. In the first strategy, the drug was linked through its carboxylic acid function to amine residues of carrier proteins (**BSA**-N-TA and casein-N-TA), while in the second strategy, the thiophene ring of TA was attached to proteins through a short 3-thiopropanoyl **linker**, the corresponding conjugates (**BSA**-S-5-TA) and beta LG-S-5-TA) thus preferentially presenting the 2,3-dichlorophenoxyacetic moiety of the drug for antibody recognition. The **BSA**-S-5-TA conjugate proved to be 30 times more immunogenic than **BSA**-N-TA. Anti-TA-protein adduct antibodies were obtained after immunization of rabbits with **BSA**-S-5-TA (1/35000 titer against beta LG-S-5-TA in ELISA). These antibodies strongly recognized the 2,3-dichlorophenoxyacetic moiety of TA but poorly the part of the drug engaged in the **covalent** binding with the proteins. This powerful tool was used in immunoblots to compare TA or TAI adduct formation in human liver microsomes as well as on microsomes from yeast expressing human liver cytochrome P450 2C9. TA displayed a highly specific **covalent** binding focused on P450 2C9 which is the main cytochrome P450 responsible for its hepatic activation in humans. On the contrary, TAI showed a nonspecific alkylation pattern, targeting many proteins upon

metabolic activation. Nevertheless, this nonspecific **covalent** binding could be completely shifted to a thiol trapping agent like GSH. The difference in alkylation patterns for these two compounds is discussed with regard to their distinct toxicities. A relationship between the specific **covalent** binding of P450 2C9 by TA and the appearance of the highly specific anti-LKM sub(2) autoantibodies (known to specifically recognize P450 2C9) in patients affected with TA-induced hepatitis is strongly suggested.

L9 ANSWER 28 OF 47 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 1998:89337 LIFESCI
TITLE: Removal of Bovine Serum Albumin from Cow's Milk Using Chicken Egg-yolk Antibodies Immobilized on Chitosan Gel
AUTHOR: Losso, J.N.; Vanderstoep, J.; Nakai, S.
CORPORATE SOURCE: Department of Food Science, University of British Columbia, 6650 NW Marine Drive, Vancouver, British Columbia, Canada V6T 1Z4
SOURCE: Food Agric. Immunol., (19980300) vol. 10, no. 1, pp. 47-56. ISSN: 0954-0105.
DOCUMENT TYPE: Journal
FILE SEGMENT: W2
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Polyclonal chicken antibodies raised against **bovine serum albumin** (**BSA**) were immobilized on chitosan gel for the immunoaffinity isolation of **BSA** from cow's milk. Antibodies (IgY) against **BSA** were isolated from egg-yolk, purified and antibody reactivity to antigen was measured. IgY developed against **BSA** was reduced by 2-mercaptoethylamine. The reactivities of reduced and whole IgY against **BSA** were not significantly different. The reduced IgY was covalently linked to chitosan gel through stable **covalent** thioether **linkages** using sulfo-succinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) as a cross-**linker**. The density of antibody IgY immobilized on chitosan gel was approximately 3-5 mg per ml of chitosan gel. The ligand-binding capacity of immobilized IgY towards **BSA** was 0.35-0.44 mg **BSA** per ml of chitosan gel. A single pass of skimmed milk through the column allowed the removal of **BSA** from the milk sample. The milk sample was analyzed, before and after immunoaffinity separation, by SDS-PAGE. **BSA** was desorbed with 0.5 M-glycine-HCl buffer at pH 2.8 but the reusability of the column was limited to three cycles. Alternatively, **BSA** was desorbed with 0.5 M-glycine-HCl buffer containing 2 M-NaCl at pH 4.6 after longer incubation times at a slower flow rate. The low ligand-binding capacity was not an impedement to reuse of the column. The column was reused more than 20 times with minimal loss of binding capacity.

L9 ANSWER 29 OF 47 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 97:77941 LIFESCI
TITLE: Mechanism for the anti-thyroid action of minocycline
AUTHOR: Doerge, D.R.; Divi, R.L.; Deck, J.; Taurog, A.
CORPORATE SOURCE: Food and Drug Admin., Natl. Cent. for Toxicol. Res., Jefferson, AR 72079-9502, USA
SOURCE: CHEM. RES. TOXICOL., (1997) vol. 10, no. 1, pp. 49-58. ISSN: 0893-228X.
DOCUMENT TYPE: Journal
FILE SEGMENT: X
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Administration of minocycline (MN), a tetracycline antibiotic, produces a black pigment in the thyroids of humans and several species of experimental animals and antithyroid effects in rodents. We have previously shown that these effects appear to be related to interactions of MN with thyroid peroxidase (TPO), the key enzyme in thyroid hormone

synthesis. In the present study, the mechanisms for inhibition of TPO-catalyzed iodination and coupling reactions by MN were investigated. MN was stable in the presence of TPO and H sub(2)O sub(2), but adding iodide or a phenolic cosubstrate caused rapid conversion to several products. TPO-dependent product formation, characterized by on-line LC-APCI/MS and super(1)H-NMR, involved oxidative elimination to form the corresponding benzoquinone with subsequent dehydrogenation at the aliphatic 4-(dimethylamino) group. Addition of thiol-containing polymers (**bovine serum albumin** or thiol-agarose chromatographic beads) had a minimal effect on MN oxidation by TPO, but substantially reduced product formation and produced concomitant losses in free thiols. **Covalent** bonding through a thioether **linkage** of a reactive intermediate, the benzoquinone iminium ion, was inferred from these findings. Iodide- and phenolic cosubstrate-dependent oxidation of tetracycline to demethylated and dehydrogenated products was also observed, although at a slower rate than MN. The products and kinetics observed with MN were consistent with oxidation of MN by either the enzymatic iodinating species formed by reaction of TPO compound I with iodide or phenoxyl radicals/cations generated by TPO-mediated oxidation of a phenolic cosubstrate. The proposed reaction mechanism is consistent with alternate substrate inhibition of TPO-catalyzed iodination of tyrosyl residues in thyroglobulin (Tg) by MN, as previously reported. Furthermore, the observed phenoxyl radical-mediated oxidation of MN is consistent with its previously reported potent inhibition of the coupling of hormonogenic iodotyrosine residues in Tg in the reaction that forms thyroid hormones. The proposed reaction mechanism also implicates a reactive benzoquinone iminium ion intermediate that could be important in toxicity of MN.

L9 ANSWER 30 OF 47 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 89:54143 LIFESCI
TITLE: The reaction of S-mercuric-N-dansylcysteine with acetylcholinesterase and butyrylcholinesterase.
AUTHOR: Tomlinson, G.
CORPORATE SOURCE: Dep. Pharmacol. and Ther., Fac. Med., 770 Bannatyne Ave., Univ. Manitoba, Winnipeg, Man. R3E 0W3, Canada
SOURCE: BIOCHEM. CELL BIOL., (1989) vol. 67, no. 7, pp. 337-344.
DOCUMENT TYPE: Journal
FILE SEGMENT: L
LANGUAGE: English
SUMMARY LANGUAGE: English; French
AB S-mercuric-N-dansylcysteine was investigated as a potential probe of protein sulphydryl groups using **bovine serum albumin**, S-carboxymethyl - **bovine serum albumin**, lysozyme, and partially reduced lysozyme as test proteins. Criteria used to assess **covalent** binding through mercury-bridged mercaptide **linkages** include a finite reaction time (minutes to hours), abolition of the characteristic fluorescence spectrum following addition of a reducing agent, and failure to separate probe and protein after chromatography or electrophoresis.

L9 ANSWER 31 OF 47 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 88:50923 LIFESCI
TITLE: Immunopotentiation of the humoral response by liposomes: Effect of a T cell polyclonal activator.
AUTHOR: Therien, H.-M.; Shahum, E.
CORPORATE SOURCE: Dep. Chim.-Biol., Univ. Quebec, C.P. 500, Trois-Rivieres, Que. G9A 5H7, Canada
SOURCE: CELL. IMMUNOL., (1988) vol. 116, no. 2, pp. 320-330.
DOCUMENT TYPE: Journal
FILE SEGMENT: F
LANGUAGE: English
SUMMARY LANGUAGE: English
AB In this paper, the authors analyzed the influence of surface-linked Con A

on the secondary response to liposome-associated antigen via either encapsulation or **covalent linkage** to the liposomal surface. The study was carried out on BALB/c mice using **bovine serum albumin** as antigen. The humoral response was evaluated by measurements of antibody-producing cells (total, IgM, and IgG) and serum antibody titers. These results suggest that the quality of an immune response and the mechanisms of activation may be profoundly influenced by the nature of antigen association with liposomes as well as by the presence at the liposomal surface of immunomodulators such as Con A.

L9 ANSWER 32 OF 47 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 88:23930 LIFESCI
TITLE: The fluid-phase binding of human C4 and its genetic variants, C4A3 and C4B1, to immunoglobulins.
AUTHOR: Kishore, N.; Shah, D.; Skanes, V.M.; Levine, R.P.
CORPORATE SOURCE: James S. McDonnell Dep. Genet., Washington Univ. Sch. Med., St. Louis, MO 63110, USA
SOURCE: MOL. IMMUNOL., (1988) vol. 25, no. 9, pp. 811-819.
DOCUMENT TYPE: Journal
FILE SEGMENT: F
LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Covalent** binding of the fourth complement protein, C4, to immune complexes is an important first step in the complement mediated processing of the complexes. The authors have characterized the **covalent** binding of C4b to immunoglobulin molecules in a fluid-phase system consisting only of antibody in solution and purified C4 and C1s. The authors demonstrate that human C4b binds to IgG in the fluid phase, that its **covalent** bindings is predominantly to the heavy chain of IgG, and that the **covalent linkage** is by either amide or acyl ester bonds. In addition, the authors compare the **covalent** binding efficiencies of two genetic variants of C4, C4A3 and C4B1, to IgG. C4A3 binds 3-4 times more IgG than C4B1 over a range of C4 concentrations, and C4A3 has a higher binding efficiency than C4B1 for IgM, IgA, IgA2a and F(ab') sub(2) as well as for a protein antigen, **BSA**. Furthermore, whereas C4A3 is bound to immunoglobulins in the fluid-phase predominantly by amide **linkage**, C4B1 is bound by either amide or acyl ester bonds. The results presented here suggest that the **covalent** binding efficiency of C4A3 and C4B1 to IgG is similar to that reported for their **covalent** binding to small molecules.

L9 ANSWER 33 OF 47 LIFESCI COPYRIGHT 2004 CSA on STN
ACCESSION NUMBER: 83:52112 LIFESCI
TITLE: Immune response mediated by liposome-associated protein antigens. III. Immunogenicity of bovine serum albumin covalently coupled to vesicle surface.
AUTHOR: Shek, P.N.; Heath, T.D.
CORPORATE SOURCE: Immunol. Group, Biosc. Div., Defence and Civil Inst. Environ. Med., Dep. Natl. Defence, Downsview, Ont. M3M 3B9, Canada
SOURCE: IMMUNOLOGY., (1983) vol. 50, no. 1, pp. 101-106.
DOCUMENT TYPE: Journal
FILE SEGMENT: F
LANGUAGE: English
SUMMARY LANGUAGE: English
AB A protein antigen, **bovine serum albumin** (**BSA**), was covalently linked to the surface of preformed large unilamella vesicles composed of phosphatidylcholine, cholesterol, and N-(4-(p-maleimidophenyl)butyryl)phosphatidylethanolamine (MPB-PE). The interaction between thiolated **BSA** and MPB-PE resulted in the production of a protein-liposome conjugate via the formation of an irreversible **covalent** bond. Mice immunized with liposome coupled **BSA** were found to generate a vigorous **BSA**-specific

plaque-forming cell (PFC) response. There seems to be a need for successful and stable **linkage** between the antigen and the carrier. The elicitation of an optimal antigen-specific PFC response was also found to require the vesicle surface to be coated with a certain minimum distribution of the antigen. Results of this study demonstrate that the **covalent** coupling of a protein antigen to the liposome surface is very effective in potentiating the protein-specific antibody response and the immunogenicity of the conjugate is dependent on the epitope density of the antigen.

L9 ANSWER 34 OF 47 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2004-0106130 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 2004 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): **Covalent** linking of proteins and cytokines to suture: Enhancing the immune response of head and neck cancer patients
AUTHOR: SHIBUYA Terry Y.; SANGHUN KIM; NGUYEN Kevin; PARIKH Parag; WADHWA Ashish; BROCKARDT Chad; DO Johnny
CORPORATE SOURCE: Departments of Otolaryngology/Head and Neck Surgery ,University of California Irvine College of Medicine, Irvine, California, United States; Chao Family Comprehensive Cancer Center , University of California Irvine Medical Center, Orange, California, United States; Boston University School of Medicine, Boston, Massachusetts, United States
SOURCE: The Laryngoscope, (2003), 113(11), 1870-1884, 133 refs.
ISSN: 0023-852X CODEN: LARYA8
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-3102, 354000118700460030

AN 2004-0106130 PASCAL
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AB Background: The immune system of advanced stage head and neck cancer patients is frequently suppressed. Poor immune function has been correlated with poor clinical outcome. Immunotherapeutic strategies have been previously attempted in an effort to enhance immune function and improve survival. Previous studies have shown surgical suture can be transformed into an immune stimulant capable of activating the T lymphocytes of cancer patients. The development of a process for covalently linking proteins and cytokines to suture could have enormous potential for the in vivo manipulation of the immune system. Hypothesis: We hypothesize proteins and cytokines can be covalently linked to surgical suture while preserving their functional properties. Study Design: Prospective study testing normal donor and head and neck squamous cell carcinoma (HNSCC) patient lymphocytes. Method: Polyester suture was acid hydrolyzed followed by reacting with 1-ethyl-3(-3-dimethylamino propyl carbodiimide) (EDAC) to create a suture-EDAC intermediate. Next, selected proteins (horseradish peroxidase [HRP] or **bovine serum albumin [BSA]**) or cytokines (interleukin [IL]-2 or interferon [IFN]-γ) were reacted with the suture-EDAC intermediate to test the **covalent linkage** of the selected protein or cytokine to suture. Functional activity of the linked proteins was measured spectrophotometrically. The linking of cytokines to suture was tested by stimulating normal donor peripheral blood lymphocytes (PBL) or HNSCC patients' lymphocytes. The functional activity was confirmed by proliferation, enzyme linked immuno-adsorbent assay (ELISA), and phenotype expression of T cells. Results: The conditions for optimally linking a protein to polyester suture were defined using HRP as a model protein. HRP retained its enzymatic

activity. The optimal conditions for linking IL-2 or IFN-γ were defined. The covalently linked cytokines retained their immune enhancing properties for stimulating PBL and lymph node lymphocytes (LNL) from HNSCC patients to proliferate, generate a T.sub.H1 immunologic profile of cytokines (IL-2, IL-12, IFN-γ), and stimulate T lymphocytes. Conclusion: This is the first report to demonstrate that cytokines can be covalently linked to surgical sutures and retain their immune-stimulating properties. Proteins linked to suture also retained their enzymatic activity. The clinical implications of functionally active cytokines or proteins linked to surgical suture may be very significant in the future for manipulating the immune system in vivo or enhancing wound healing.

L9 ANSWER 35 OF 47 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2001-0280431 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 2001 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): Stabilization of penicillin V acylase from Streptomyces lavendulae by **covalent** immobilization
AUTHOR: TORRES-BACETE Jesus; ARROYO Miguel; TORRES-GUZMAN Raquel; DE LA MATA Isabel; CASTILLON M. Pilar; ACEBAL Carmen
CORPORATE SOURCE: Departamento de Bioquimica y Biologia Molecular I, Facultad de Ciencias Biologicas, Universidad Complutense de Madrid, 28040 Madrid, Spain
SOURCE: Journal of chemical technology and biotechnology : (1986), (2001), 76(5), 525-528, 26 refs.
ISSN: 0268-2575 CODEN: JCTBDC
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United Kingdom
LANGUAGE: English
AVAILABILITY: INIST-560, 354000095273270130

AN 2001-0280431 PASCAL
CP Copyright .COPYRGT. 2001 INIST-CNRS. All rights reserved.
AB Penicillin V acylase from the actinomycete Streptomyces lavendulae ATCC 13664 has been immobilized to epoxy-activated acrylic beads (Eupergit C.sup.R) by **covalent** binding. Further **linkage** of **bovine serum albumin** after enzyme immobilization was carried out in order to remove the remaining oxirane groups of the support. The obtained immobilized biocatalyst displayed double exponential deactivation kinetics at temperatures below 55 °C, while the native enzyme followed single exponential decay at the same temperatures. We concluded that soluble penicillin acylase was deactivated in one step, whereas the immobilized enzyme showed an enzymatic intermediate state which is highly thermostable. As a consequence of the immobilization process, the enzyme displayed a 10-fold increase in its half-life at 40 °C. At this temperature, the enzymatic intermediate state was progressively destabilized as the pH of the medium was increased. Thus, the optimum pH range for the immobilized enzyme preparation was established as being from 7.0 to 8.0. Higher pH values led to quicker enzyme deactivation.

L9 ANSWER 36 OF 47 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 2000-0452131 PASCAL
TITLE (IN ENGLISH): Investigation into immobilisation of lactate oxidase to improve stability
AUTHOR: LILLIS B.; GROGAN C.; BERNEY H.; LANE W. A.
CORPORATE SOURCE: Natl Microelectronics Research Cent, Cork, Ireland
SOURCE: Sensors and Actuators, B: Chemical, (2000), 68(1), 109-114, 15 refs.
Conference: Proceedings of Eurosensors XIII, The

Hague, Neth, 12 Sep 1999-15 Sep 1999
ISSN: 0925-4005
DOCUMENT TYPE: Journal; Conference
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: Switzerland
LANGUAGE: English
AVAILABILITY: INIST-19425 B
AN 2000-0452131 PASCAL
AB Lactate oxidase (LOx) is an unstable enzyme. In this work, a variety of immobilisation techniques are investigated in an effort to improve the long-term stability of the enzyme. These include **covalent linkage** to two membrane types, encapsulation in a **BSA** gel and four different sol-gel matrices. The enzyme glucose oxidase (GOx) was also immobilised in the same sol-gel matrices. The methods were assessed for both activity and stability of the enzyme and the mechanical rigidity of the matrix. The **BSA** and sol-gels both formed physically robust enzyme layers. The enzyme retained its activity in the **BSA** gel for 20 days. Activity of the enzyme was much higher in the sol-gel matrices and remained stable for at least 55 days. Sol-gel processing conditions were also investigated.

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ACCESSION NUMBER: 2000-0309920 PASCAL
TITLE (IN ENGLISH): Protein immobilization to a partially cross-linked organic monolayer
AUTHOR: VIITALA T.; VIKHOLM I.; PELTONEN J.
CORPORATE SOURCE: Abo Akademi Univ, Turku, Finland
SOURCE: Langmuir, (2000), 16(11), 4953-4961, 51 refs.
ISSN: 0743-7463
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-20642
AN 2000-0309920 PASCAL
AB The **covalent** attachment of Fab$PRM fragments of polyclonal anti-human IgG to a polymerizable lipid with a terminal **linker** group was examined by means of quartz crystal microbalance (QCM), surface plasmon resonance (SPR), and atomic force microscopy (AFM). The **linker** lipid was embedded in a monolayer of dilinoeloylphosphatidylethanolamine. Both monomeric and cross-linked biofunctionalized monolayers were studied. Atomic force microscope images showed that the monomeric monolayer consisted of large holes when it was deposited on a solid substrate, while the cross-linked monolayer appeared as a planar two-dimensional film. The ability of the biofunctionalized monolayer to bind proteins decreased with UV-irradiation time. However, an increase in the **linker** lipid concentration in the lipid matrix increased the protein-binding efficiency. A comparison between QCM and SPR measurements indicated that the QCM measurements overestimated the binding efficiency of immobilized Fab$PRM fragments toward hIgG. AFM images visualized the topographical changes of the different stages of the monolayer incubation in Fab$PRM, **BSA**, and hIgG protein solutions.

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ACCESSION NUMBER: 1999-0375635 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 1999 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): An immunochemical study on tau glycation in paired helical filaments
AUTHOR: KO L.-W.; KO E. C.; NACHARAJU P.; LIU W.-K.; CHANG E.; KENESSEY A.; YEN S.-H. C.

CORPORATE SOURCE: Department of Pathology, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461, United States; Mayo Clinic Jacksonville, Birdsall Building, Room 357, 4500 San Pablo Road, Jacksonville, FL 32224, United States
SOURCE: Brain research, (1999), 830(2), 301-313, 74 refs.
ISSN: 0006-8993 CODEN: BRREAP
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: Netherlands
LANGUAGE: English
AVAILABILITY: INIST-12895, 354000085403540110
AN 1999-0375635 PASCAL

AB Glycation is a non-enzymatic posttranslational modification that involves a **covalent linkage** between a sugar and an amino group of protein molecule forming ketoamine. Subsequent oxidation, fragmentation and/or crosslinking of ketoamine leads to the production of advanced glycation endproducts (AGEs). Formation of AGEs causes detrimental effects on the structure and function of affected proteins. Accumulation of AGEs has been implicated in normal aging and in the pathogenesis of diabetes-associated complications and Alzheimer's disease (AD). Of all AGEs, N.sup.ε-(carboxymethyl)lysine (CML) is a major glycoxidation product known to be stable and accumulate progressively in vivo. In order to determine if tau is glycated in AD, we raised a rabbit antibody to CML that demonstrated its usefulness in detecting glycation of different proteins in vitro, including **BSA**, ribonuclease, lysozyme and recombinant tau. Immunochemical analyses indicated that ribose and glucose-6-phosphate are more effective than glucose in generating CML formation in these proteins. We used this antibody to probe for glycation in the following human tau preparations: tau of normal brains and preparations of soluble PHF-tau as well as insoluble PHF from AD brains. All three principal tau components resolved from PHF-tau on Western blots showed CML immunoreactivity indicating that tau is glycated in PHF-tau; and insoluble PHF exhibited prominent CML immunoreactivity on top of the stacking gel. Moreover, immunoelectron microscopic analyses indicate that the anti-CML antibody labels predominantly PHF in aggregates. Taken together, these results suggest that tau becomes glycated in PHF-tau and glycation may play a role in stabilizing PHF aggregation leading to tangle formation in AD.

L9 ANSWER 39 OF 47 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1999-0067486 PASCAL

TITLE (IN ENGLISH): In situ quartz crystal microbalance monitoring of Fab'-fragment binding to **linker** lipids in a phosphatidylcholine monolayer matrix. Application to immunosensors
AUTHOR: VIKHOLM I.; ALBERS W. M.; VAELIMAEKI H.; HELLE H. STROEVE Pieter (pref.)
CORPORATE SOURCE: Technical Research Centre of Finland, Chemical Technology, P.O. Box 14021, 33101 Tampere, Finland Center on Polymer and Macromolecular Assemblies, Department of Chemical Engineering and Materials Science, University of California, Davis, CA, United States
SOURCE: Thin solid films, (1998), 327-29, 643-646, 18 refs.
Conference: 8 International Conference on Organized Molecular Films, Pacific Grove CA (United States), 24 Aug 1997
ISSN: 0040-6090 CODEN: THSFAP
DOCUMENT TYPE: Journal; Conference

BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: Switzerland
LANGUAGE: English
AVAILABILITY: INIST-13597, 354000071007471450
AN 1999-0067486 PASCAL

AB **Linker** lipids were embedded in a phosphatidylcholine monolayer matrix prepared at the air-water interface. The **covalent** coupling of antibody fragments, non-specific adsorption of **bovine serum albumin** and specific binding of antibodies was monitored in situ with a 10-MHz quartz crystal microbalance. The attachment of antibody fragments and the activity of the layers was also showed with standardized radioimmunoassay. The results demonstrate that the coupling of Fab'-fragments to **linker** lipids in a monolayer matrix is a promising approach to achieve a highly oriented layer of antibody fragments with a high density of binding sites on the sensor surface for immunological measurements.

L9 ANSWER 40 OF 47 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1998-0442687 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 1998 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): Oriented immobilization of antibodies for immunosensing
AUTHOR: VIKHOLM I.; ALBERS W. M.
CORPORATE SOURCE: Technical Research Centre of Finland, Chemical Technology, P.O. Box 14021, 33101 Tampere, Finland
SOURCE: Langmuir, (1998), 14(14), 3865-3872, 52 refs.
ISSN: 0743-7463 CODEN: LANGD5
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-20642, 354000077129050200
AN 1998-0442687 PASCAL

AB The **covalent** coupling of antibody fragments to **linkers** embedded in a monolayer matrix of phosphatidylcholine and cholesterol was examined at the air-water interface by the means of a quartz crystal microbalance, QCM. Two **linkers** that bind the free thiols of the Fab' fragment were investigated. The nonspecific binding of **bovine serum albumin** and the specific binding of antigen were also monitored with the QCM. Standardized radioimmunoassay was used to confirm the immunoreaction and determine binding parameters. The monolayer formation of the **linker** lipids in the ternary system of phosphatidylcholine and cholesterol was, moreover, demonstrated by film balance studies. The results demonstrate that the **covalent** coupling of Fab' fragments to linking groups embedded in a phospholipid monolayer matrix is a promising approach to achieve a defined immobilization of antibodies at the sensor surface with high antigen binding effeciency.

L9 ANSWER 41 OF 47 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1998-0284900 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 1998 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): Removal of **bovine serum albumin** from cow's milk using chicken egg-yolk antibodies immobilized on chitosan gel
AUTHOR: LOSSO J. N.; VANDERSTOEP J.; NAKAI S.
CORPORATE SOURCE: Department of Food Science, University of British Columbia, 6650 NW Marine Drive, Vancouver, British

Columbia, V6T 1Z4, Canada
SOURCE: Food and agricultural immunology, (1998), 10(1), 47-56, 19 refs.
ISSN: 0954-0105
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United Kingdom
LANGUAGE: English
AVAILABILITY: INIST-26427, 354000075919290060
AN 1998-0284900 PASCAL

AB Polyclonal chicken antibodies raised against **bovine serum albumin** (**BSA**) were immobilized on chitosan gel for the immunoaffinity isolation of **BSA** from cow's milk. Antibodies (IgY) against **BSA** were isolated from egg-yolk, purified and antibody reactivity to antigen was measured. IgY developed against **BSA** was reduced by 2-mercaptoethylamine. The reactivities of reduced and whole IgY against **BSA** were not significantly different. The reduced IgY was covalently linked to chitosan gel through stable **covalent** thioether **linkages** using sulfo-succinimidyl-4-(N-maleimidomethyl)cyrlohexaiie-1-carboxylate (sulfo-SMCC) as a cross-**linker**. The density of antibody IgY immobilized on chitosan gel was approximately 3-5 mg per ml of chitosan gel. The ligand-binding capacity of immobilized IgY towards **BSA** was 0.35-0.44 mg **BSA** per ml of chitosan gel. A single pass of skimmed milk through the column allowed the removal of **BSA** from the milk sample. The milk sample was analyzed, before and after immunoaffinity separation, by SDS-PAGE. **BSA** was desorbed with 0.5 M-glycine-HCl buffer at pH 2.8 but the reusability of the column was limited to three cycles. Alternatively, **BSA** was desorbed with 0.5 M-glycine-HCl buffer containing 2 M-NaCl at pH 4.6 after longer incubation time at a slower flow rate. The low ligand-binding capacity was not an impedement to reuse of the column. The column was reused more than 20 times with minimal loss of binding capacity.

L9 ANSWER 42 OF 47 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1998-0038535 PASCAL

TITLE (IN ENGLISH): Chemical and physical characterization of a novel poly(carbonate urea) urethane surface with protein crosslinker sites
AUTHOR: PHANEUF M. D.; QUIST W. C.; LOGERFO F. W.; SZYCHER M.; DEMPSEY D. J.; BIDE M. J.
CORPORATE SOURCE: Beth Israel Deaconess Medical Center/Harvard Medical School, Department of Surgery, 4 Blackfan Circle, H.I.M. Building, Room 131, Boston, MA 02115, United States; Beth Israel Deaconess Medical Center/Harvard Medical School, Department of Pathology, 4 Blackfan Circle, H.I.M. Building, Room 131, Boston, MA 02115, United States; CardioTech International, Inc., 11 State Street, Woburn, MA 01801, United States; University of Rhode Island, Department of Textiles, 311 Quinn Hall, Kingston, RI 02281, United States
SOURCE: Journal of biomaterials applications, (1997), 12(2), 100-120, 66 refs.
ISSN: 0885-3282 CODEN: JBAPEL
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-21113, 354000079614030010
AN 1998-0038535 PASCAL

CP Copyright .COPYRGT. 1998 INIST-CNRS. All rights reserved.
AB A major complication which occurs with implantable polyurethane biomaterials is bioincompatibility between blood and the biomaterial surface. Development of a novel biodurable polyurethane surface to which biological agents, such as growth factors or anticoagulants could be covalently bound, would be beneficial. The purpose of this study was to synthesize a novel poly(carbonate urea) urethane polymer with carboxylic acid groups which would serve as "anchor" sites for protein attachment. Physical characteristics such as tensile strength, initial modulus, ultimate elongation, tear strength, water/alcohol uptake and water vapor permeation were then evaluated and compared to other biomedical-grade polyurethanes. **Covalent linkage** of the blood protein albumin to this novel surface was then examined. A biodurable polycarbonate-based polyurethane containing carboxylic acid groups (cPU) was synthesized using a two step procedure incorporating the chain extender 2,2-bis(hydroxymethyl)-propionic acid (DHMPA). Tensile strength of this cPU film was 2.7 and 2.6 fold greater than both a polycarbonate-based polyurethane synthesized with a 1,4-butanediol chain extender (bdPU) and Mitrathane (Mit) controls, respectively. The cPU polymer also possessed 7.8 and 31 fold greater structural rigidity upon evaluation of initial modulus as compared to the bdPU and Mit, respectively. Ultimate elongation for the bdPU films was slightly higher than the cPU and Mit films, which had comparable elongation properties. The force required to tear the bdPU film was 1.9 and 32 fold greater than the cPU and Mit films, respectively. Alcohol solution uptake by all of the polyurethane segments increased with increasing alcohol concentrations, with the cPU having the greatest uptake. Water uptake was minimal for all the polyurethanes examined and was not affected by altering pH. Water vapor permeation was lowest for the cPU films as compared to both bdPU and Mit. Swelling the cPU in 50% ethanol prior to evaluation slightly increased water vapor permeation through the films. **Covalent linkage** of the radiolabelled blood protein albumin (.sup.1.sup.2.sup.5I-**BSA**) to the cPU segments incubated with the heterobifunctional crosslinker 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was greatest in the higher percent of ethanol as compared to controls. These results serve as foundation for developing a novel poly(carbonate urea) urethane with physical characteristics comparable to other medical-grade polyurethanes while having protein binding capabilities.

L9 ANSWER 43 OF 47 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN

ACCESSION NUMBER: 1997-0320904 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 1997 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): **Covalent linkage** of recombinant hirudin to poly(ethylene terephthalate) (Dacron) : creation of a novel antithrombin surface
AUTHOR: PHANEUF M. D.; BERCELI S. A.; BIDE M. J.; QUIST W. C.; LOGERFO F. W.
CORPORATE SOURCE: Deaconess Hospital/Harvard Medical School, Vascular Surgery Research, 12 Blackfan Street, Boston, MA 02215, United States; University of Rhode Island, Department of Textiles, 311 Ouinn Hall, Kingston, RI 02881, United States
SOURCE: Biomaterials, (1997), 18(10), 755-765, 49 refs. ISSN: 0142-9612
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United Kingdom
LANGUAGE: English
AVAILABILITY: INIST-18773, 354000065732250080
AN 1997-0320904 PASCAL
CP Copyright .COPYRGT. 1997 INIST-CNRS. All rights reserved.

AB Thrombus formation and intimal hyperplasia on the surface of implantable biomaterials such as poly(ethylene terepthalate) (Dacron) vascular grafts are major concerns when utilizing these materials in the clinical setting. Thrombin, a pivotal enzyme in the blood coagulation cascade primarily responsible for thrombus formation and smooth muscle cell activation, has been the target of numerous strategies to prevent this phenomenon from occurring. The purpose of this study was to covalently immobilize the potent, specific antithrombin agent recombinant hirudin (rHir) to a modified Dacron surface and characterize the in vitro efficacy of thrombin inhibition by this novel biomaterial surface. **Bovine serum albumin** (**BSA**), which was selected as the 'basecoat' protein, was reacted with various molar ratios of the cross-**linker** sulphosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulpho-SMCC; 1 :5-1 :50). These **BSA**-SMCC complexes were then covalently linked to sodium hydroxide-hydrolysed Dacron (HD) segments via the cross-**linker** 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC). **Covalent linkage** of these complexes to HD (HD-**BSA**-SMCC) was not affected by any of the sulpho-SMCC cross-**linker** ratios assayed. rHir, which was initially reacted with 2-iminothiolane hydrochloride (Traut's reagent) in order to create sulphydryl groups, was then covalently bound to these HD-**BSA**-SMCC surfaces (HD-**BSA**-SMCC-S-rHir). The 1:50 (**BSA**: sulpho-SMCC) HD-**BSA**-SMCC-S-rHir segments bound 22-fold more rHir (111 ng per mg Dacron) compared to control segments and also possessed the greatest thrombin inhibition of the segments evaluated using a chromogenic substrate assay for thrombin. Further characterization of the HD-**BSA**-SMCC-S-rHir segments demonstrated that maximum thrombin inhibition was 20.43 NIHU, 14.6-fold greater inhibition than control segments (1.4 NIHU). Thrombin inhibition results were confirmed by .sup.1.sup.2.sup.5I-thrombin binding experiments, which demonstrated that the 1:50 HD-**BSA** -SMCC-S-rHir segments had significantly greater specific thrombin adhesion compared to control segments. Non-specific .sup.1.sup.2.sup.5I-thrombin binding to and release from the 1:50 HD-**BSA** -SMCC-S-rHir segments was also significantly less than the control segments. Thus, these results demonstrate that rHir can be covalently bound to a clinically utilized biomaterial (Dacron) while still maintaining its ability to bind and inhibit thrombin.

L9 ANSWER 44 OF 47 PASCAL on STN

ACCESSION NUMBER: 1996-0283888 PASCAL

TITLE (IN ENGLISH): Preparative membrane adsorber chromatography for the isolation of cow milk components

AUTHOR: SPLITT H.; MACKENSTEDT I.; FREITAG R.
LINDNER W. (ed.)

CORPORATE SOURCE: Institut fuer Technische Chemie, Callinstr. 3, 30167 Hannover, Germany, Federal Republic of
Institute of Pharmaceutical Chemistry, Karl Franzens University of Graz, 8010 Graz, Austria
Austrian Society of Analytical Chemistry ASAC, Austria (patr.); Austrian Chemical Society GOECh, Austria (patr.)

SOURCE: Journal of chromatography. A, (1996), 729(1-2), 87-98 [11 p.], 32 refs.
Conference: 19 HPLC'95 : International Symposium on Column Liquid Chromatography and Related Techniques, Innsbruck (Austria), 28 May 1995

DOCUMENT TYPE: Journal; Conference

BIBLIOGRAPHIC LEVEL: Analytic

COUNTRY: Netherlands

LANGUAGE: English
AVAILABILITY: INIST-8577A, 354000043253960110
AN 1996-0283888 PASCAL

AB Preparative membrane adsorber (MA) chromatography was used to process milk fractions such as the whey and the permeate commonly obtained during lactose production in modern dairies. In MA systems the fluid-dynamic and mass-transfer properties are superior to conventional HPLC or fast protein liquid chromatography (FPLC) columns. Since the flow resistance caused by the MA stacks is quite low, high throughputs can be realized without loss in resolution. Feed sizes were varied from the laboratory scale (several ml) up to batches of 10 l during the investigations. MAs based on modified cellulose filtration membranes (average single layer thickness 200 μm, average pore size 5.0 μm) were used for the small-scale experiments. The MAs are functionalized by **covalent linkage** of strong and weak ion exchanger groups to their surface. Three commercially available types were used [strong ion exchanger : MA Q15 (3 layers of 5 cm.sup.2) and MA Q100 (5 layers of 20 cm.sup.2) ; weak ion exchanger : MA D15 (3 layers of 5 cm.sup.2) ; all Sartorius, Germany]. For the large-scale work a dead-end filtration unit containing up to 1300 cm.sup.2 of MA-area was used. Here MAs based on a synthetic copolymer, that were prepared from cut-out sheets, were inserted. Chromatographic conditions were transferable from the cellulose- to the polymer-based MA carrying the same functional groups. The influence of the flow-rate and the pH of the mobile phase on the separation was investigated. The flow-rate could be raised to the limit of the respective chromatographic systems and/or MA modules without loss in resolution. The use of the strong anion exchanger MA together with a mobile phase pH of 6.0 and a fine-tuned gradient allowed the separation of **BSA**, α-lactalbumin and the genetic variants of β-lactoglobulin, even though no baseline separation was possible in the latter case. The use of coupled modules rather than a single one is shown to improve the separation considerably.

L9 ANSWER 45 OF 47 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 1995-0147511 PASCAL

TITLE (IN ENGLISH): Aggregation of proteins and its prevention by carbohydrate excipients : albumins and γ-globulin
AUTHOR: MANOHAR KATAKAM; BANGA A. K.
CORPORATE SOURCE: Auburn univ., school pharmacy, dep. pharmacal sci., Auburn AL 36849-5503, United States
SOURCE: Journal of pharmacy and pharmacology, (1995), 47(2), 103-107, 13 refs.
ISSN: 0022-3573 CODEN: JPPMAB
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United Kingdom
LANGUAGE: English
AVAILABILITY: INIST-984, 354000059636820020
AN 1995-0147511 PASCAL

AB Moisture-induced (2-10 μL added to 10 mg) aggregation of solid-state albumin and γ-globulin was investigated by incubation at 37.sup.oC for 24 h. The insoluble aggregates were centrifuged from a reconstituted solution, dissolved in a solution containing denaturant and reducing agent, and analysed by a Bio-Rad protein assay kit. Of the three albumins used, maximum aggregation (8.2%) was observed with **bovine serum albumin** that was essentially fatty-acid free. The maximum aggregation observed with γ-globulin was 7.0%. A bell-shaped curve for percent aggregation was observed with increasing

moisture content and was especially prominent for **bovine serum albumin**. When mixed with carbohydrate excipients in a 1:1 ratio, aggregation was reduced for both **bovine serum albumin** and γ-globulin by all four of the following excipients used: Emdex, dextrose, trehalose and hydroxypropyl β-cyclodextrin. For **bovine serum albumin**, the aggregation was reduced about sixfold, with Emdex being the most effective excipient. The likely mechanism of the resulting aggregation was **covalent linkages** formed due to intermolecular thiol disulphide interchange

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ACCESSION NUMBER: 1993-0108816 PASCAL
TITLE (IN ENGLISH): Microporous poly (caprolactam) hollow fibers for therapeutic affinity adsorption
AUTHOR: KUGEL K.; MOSELEY A.; HARDING G. B.; KLEIN E.
CORPORATE SOURCE: Univ. Louisville, school medicine, kidney disease program, Louisville KY 10292, United States
SOURCE: Journal of membrane science, (1992), 74(1-2), 115-129, 18 refs.
ISSN: 0376-7388 CODEN: JMESDO
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: Netherlands
LANGUAGE: English
AVAILABILITY: INIST-17232, 354000030776830110

AN 1993-0108816 PASCAL
AB Microporous nylon-6 hollow fibers were modified for use as affinity fibers. The initial amine end-group concentration of 21 μmoles/g fiber was amplified by reacting the polymer withlysine, using stanard peptide synthetic method. The carbohydrate side chain of a rabbit polyclonal anti-**BSA** IgG was oxidized to facilitate **linkage** of the Ab to the hollow fibers. The **covalent** links were either to terminal amine groups [from lysine of the poly(caprolactam)] or to hydrazide groups. The latter were produced by coupling adipic acid dihydrazide to the amine groups via a glutaraldehyde bridge

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ACCESSION NUMBER: 1991-0226729 PASCAL
TITLE (IN ENGLISH): Liposomal vaccine : influence of antigen association on the kinetics of the humoral response
AUTHOR: THERIEN H.-M.; LAIR D.; SHAHUM E.
CORPORATE SOURCE: Univ. Quebec Trois-Rivieres, dep. chimie-biologie, Trois-Rivieres PQ G9A 5H7, Canada
SOURCE: Vaccine, (1990), 8(6), 558-562, 24 refs.
ISSN: 0264-410X CODEN: VACCDE
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United Kingdom
LANGUAGE: English
AVAILABILITY: INIST-20289, 354000018084020080

AN 1991-0226729 PASCAL
AB From our data it can be concluded that surface **linkage** is the best way to induce a rapid, intense and prolonged response which, in contrast to that induced by encapsulated **BSA**, is characterized by a low IgG/IgM ratio. The results are discussed in relation to the possible routes followed by the antigen depending on its mode of association with liposomes in the initiation of the humoral response